

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

| | | | |
|---|--|---------------------------|--|
| (51) International Patent Classification ⁶ : | | A2 | (11) International Publication Number: WO 97/07198 |
| C12N | | | (43) International Publication Date: 27 February 1997 (27.02.97) |
| | | | |
| (21) International Application Number: | | PCT/US96/12897 | |
| (22) International Filing Date: | | 8 August 1996 (08.08.96) | |
| (30) Priority Data: Not furnished | | 11 August 1995 (11.08.95) | US |
| | | | |
| (71) Applicant: GENETICS INSTITUTE, INC. [US/US]; 87 CambridgePark Drive, Cambridge, MA 02140 (US). | | | |
| | | | |
| (72) Inventors: JACOBS, Kenneth; 151 Beaumont Avenue, Newton, MA 02160 (US). MCCOY, John, M.; 63 Pine Ridge Road, Reading, MA 01867 (US). KELLEHER, Kerry; 50 Hurley Circle, Marlborough, MA 01752 (US). CARLIN, McKeough; 16 Chauncy Street #22, Cambridge, MA 02138 (US). | | | |
| | | | |
| (74) Agent: BROWN, Scott, A.; Genetics Institute, Inc., Legal Affairs, 87 CambridgePark Drive, Cambridge, MA 02140 (US). | | | |
| | | | |

(81) Designated States: AU, CA, JP, MX, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published

Without international search report and to be republished upon receipt of that report.

(54) Title: DNA SEQUENCES AND SECRETED PROTEINS ENCODED THEREBY

(57) Abstract

Novel polynucleotides and the proteins encoded thereby are disclosed.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

| | | | | | |
|----|--------------------------|----|--|----|--------------------------|
| AM | Armenia | GB | United Kingdom | MW | Malawi |
| AT | Austria | GE | Georgia | MX | Mexico |
| AU | Australia | GN | Guinea | NE | Niger |
| BB | Barbados | GR | Greece | NL | Netherlands |
| BE | Belgium | HU | Hungary | NO | Norway |
| BF | Burkina Faso | IE | Ireland | NZ | New Zealand |
| BG | Bulgaria | IT | Italy | PL | Poland |
| BJ | Benin | JP | Japan | PT | Portugal |
| BR | Brazil | KE | Kenya | RO | Romania |
| BY | Belarus | KG | Kyrgyzstan | RU | Russian Federation |
| CA | Canada | KP | Democratic People's Republic of Korea | SD | Sudan |
| CF | Central African Republic | KR | Republic of Korea | SE | Sweden |
| CG | Congo | KZ | Kazakhstan | SG | Singapore |
| CH | Switzerland | LJ | Liechtenstein | SI | Slovenia |
| CI | Côte d'Ivoire | LK | Sri Lanka | SK | Slovakia |
| CM | Cameroon | LR | Liberia | SN | Senegal |
| CN | China | LT | Lithuania | SZ | Swaziland |
| CS | Czechoslovakia | LU | Luxembourg | TD | Chad |
| CZ | Czech Republic | LV | Latvia | TG | Togo |
| DE | Germany | MC | Monaco | TJ | Tajikistan |
| DK | Denmark | MD | Republic of Moldova | TT | Trinidad and Tobago |
| EE | Estonia | MG | Madagascar | UA | Ukraine |
| ES | Spain | ML | Mali | UG | Uganda |
| FI | Finland | MN | Mongolia | US | United States of America |
| FR | France | MR | Mauritania | UZ | Uzbekistan |
| GA | Gabon | | | VN | Viet Nam |

DNA SEQUENCES AND SECRETED PROTEINS ENCODED THEREBY

5 This application claims priority from application Ser. No. 08/514,014, filed on August 11, 1995, which was converted to provisional application Ser. No. 60/_____ on July 19, 1996.

FIELD OF THE INVENTION

10 The present invention provides novel polynucleotides and proteins encoded by such polynucleotides, along with therapeutic, diagnostic and research utilities for these polynucleotides and proteins.

BACKGROUND OF THE INVENTION

15 Technology aimed at the discovery of protein factors (including e.g., cytokines, such as lymphokines, interferons, CSFs and interleukins) has matured rapidly over the past decade. The now routine hybridization cloning and expression cloning techniques clone novel polynucleotides "directly" in the sense that they rely on information directly related to the discovered factor (i.e., partial DNA/amino acid sequence of the factor in the case of hybridization cloning; activity of the factor in the case of expression cloning). More recent "indirect" cloning techniques such as signal sequence cloning, which isolates DNA sequences based on the presence of a now well-recognized secretory leader sequence motif, as well as various PCR-based or low stringency hybridization cloning techniques, have advanced the state of the art by 20 making available large numbers of DNA/amino acid sequences for factors that are known to have biological activity by virtue of their secreted nature in the case of leader sequence cloning, or by virtue of the cell or tissue source in the case of PCR-based techniques. It is to these factors and the polynucleotides encoding them that the 25 present invention is directed.

30

SUMMARY

In one embodiment, the present invention provides a composition comprising an isolated polynucleotide selected from the group consisting of:

- (a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:1 from nucleotide 38 to nucleotide 1447;
- 10 (b) a polynucleotide comprising a fragment of the nucleotide sequence of SEQ ID NO:1 encoding a protein having biological activity;
- (c) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:2;
- 15 (d) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:2 having biological activity;
- (e) a polynucleotide which is an allelic variant of SEQ ID NO:1; and
- 20 (f) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(e).

In another embodiment, the present invention provides a composition comprising an isolated polynucleotide selected from the group consisting of:

- (a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:3 from nucleotide 52 to nucleotide 2034;
- 25 (b) a polynucleotide comprising a fragment of the nucleotide sequence of SEQ ID NO:3 encoding a protein having biological activity;
- (c) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:4;
- (d) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:4 having biological activity;
- 30 (e) a polynucleotide which is an allelic variant of SEQ ID NO:4; and
- (f) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(e).

In another embodiment, the present invention provides a composition comprising an isolated polynucleotide selected from the group consisting of:

5 (a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:5 from nucleotide 76 to nucleotide 474;

10 (b) a polynucleotide comprising a fragment of the nucleotide sequence of SEQ ID NO:5 encoding a protein having biological activity;

15 (c) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:6;

20 (d) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:6 having biological activity;

25 (e) a polynucleotide which is an allelic variant of SEQ ID NO:5; and

30 (f) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(e).

In another embodiment, the present invention provides a composition comprising an isolated polynucleotide selected from the group consisting of:

35 (a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:7 from nucleotide 67 to nucleotide 348;

40 (b) a polynucleotide comprising a fragment of the nucleotide sequence of SEQ ID NO:7 encoding a protein having biological activity;

45 (c) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:8;

50 (d) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:8 having biological activity;

55 (e) a polynucleotide which is an allelic variant of SEQ ID NO:7; and

60 (f) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(e).

In another embodiment, the present invention provides a composition comprising an isolated polynucleotide selected from the group consisting of:

65 (a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:9 from nucleotide 75 to nucleotide 356;

70 (b) a polynucleotide comprising a fragment of the nucleotide sequence of SEQ ID NO:9 encoding a protein having biological activity;

- 5 (c) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:10;

(d) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:10 having biological activity;

(f) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(e).

In another embodiment, the present invention provides a composition comprising an isolated polynucleotide selected from the group consisting of:

15 (a) a polynucleotide comprising the nucleotide sequence of SEQ
ID NO:11 from nucleotide 86 to nucleotide 544;

(b) a polynucleotide comprising a fragment of the nucleotide sequence of SEQ ID NO:11 encoding a protein having biological activity.

(b) a polynucleotide comprising a fragment of the nucleotide sequence of SEQ ID NO:11 encoding a protein having biological activity;

20 (c) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:12;

(d) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:12 having biological activity;

(e) a polynucleotide which is an allelic variant of SEQ ID NO:11;
and

(f) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(e).

In certain preferred embodiments, the polynucleotide is operably linked to an expression control sequence. The invention also provides a host cell, including bacterial, yeast, insect and mammalian cells, transformed with such polynucleotide compositions.

Processes are also provided for producing a protein, which comprise:

(a) growing a culture of the host cell transformed with such

(b) purifying the protein from the culture

35 The protein produced according to such methods is also provided by the present

5 Compositions comprising a protein biological activity are also disclosed. In preferred embodiments the protein comprises an amino acid sequence selected from the group consisting of:

- (a) the amino acid sequence of SEQ ID NO:2;
- (b) fragments of the amino acid sequence of SEQ ID NO:2;
- 10 (c) the amino acid sequence of SEQ ID NO:4;
- (d) fragments of the amino acid sequence of SEQ ID NO:4;
- (e) the amino acid sequence of SEQ ID NO:6;
- (f) fragments of the amino acid sequence of SEQ ID NO:6;
- (g) the amino acid sequence of SEQ ID NO:8;
- 15 (h) fragments of the amino acid sequence of SEQ ID NO:8;
- (i) the amino acid sequence of SEQ ID NO:12; and
- (j) fragments of the amino acid sequence of SEQ ID NO:12;

the protein being substantially free from other mammalian proteins.

Such compositions may further comprise a pharmaceutically acceptable carrier.
20 Compositions comprising an antibody which specifically reacts with such protein are also provided by the present invention.

Methods are also provided for preventing, treating or ameliorating a medical condition which comprises administering to a mammalian subject a therapeutically effective amount of a composition comprising a protein of the present invention and
25 a pharmaceutically acceptable carrier.

BRIEF DESCRIPTION OF FIGURES

Fig. 1 is an autoradiograph evidencing the expression of clone J5 in COS cells (indicated by arrows). J5 is processed into multiple bands, with the major band at
30 approximately 58 kD.

Fig. 2 is an autoradiograph evidencing the expression of clone L105 in COS cells (indicated by arrows).

Fig. 3 is an autoradiograph evidencing the expression of clone H174 in COS cells (indicated by arrows).

35 Fig. 4 is an autoradiograph evidencing the expression of clone B18 in COS cells (indicated by arrows).

DETAILED DESCRIPTIONISOLATED PROTEINS AND POLYNUCLEOTIDES

The sequence of a polynucleotide encoding one protein of the present invention is set forth in SEQ ID NO:1, with the coding region extending from nucleotides 38 to 1447. This polynucleotide has been identified as "clone J5". The amino acid sequence of the protein encoded by clone J5 is set forth in SEQ ID NO:2. Clone J5 was deposited with the American Type Culture Collection on August 11, 1995 and given the accession number ATCC 69885. SEQ ID NO:1 represents a spliced combination of sequence obtained from an isolated clone identified as "J5_3.fl", with additional 5' sequence obtained from a second double stranded clone. Clone J5 was isolated from a human activated peripheral blood mononuclear cell (PBMC) library using a trap which selects for nucleotides encoding secreted proteins; therefore, clone J5 does encode a secreted factor. J5 encodes a novel protein; BLASTN/BLASTX or FASTA searches revealed no exact sequence matches. However, a BLASTX search revealed homology between the J5 protein (in the approximate region of amino acids 62-129 of SEQ ID NO:2), epididymal apical proteins (including without limitation, epididymal apical protein I-precursor (*Macaca fascicularis*) (accession X66139)) and several snake venom haemorrhagic peptides (disintegrins) (including without limitation those assigned accession U01235-1237, X68251, and M89784). Analysis of the full-length J5 sequences revealed that the disintegrin domain was incomplete and that this clone did not contain an EGF-domain, as with some of the other disintegrin family members. J5 does contain a conserved metallo-proteinase domain. Based upon these homologies, J5 and these homologous proteins are expected to share at least some activities.

The sequence of a polynucleotide encoding another protein of the present invention is set forth in SEQ ID NO:3, with the coding region extending from nucleotides 52 to 2034. This polynucleotide has been identified as "clone J422". The amino acid sequence of the protein encoded by clone J422 is set forth in SEQ ID NO:4. Clone J422 was deposited with the American Type Culture Collection on August 11, 1995 and given the accession number ATCC 69884. SEQ ID NO:3 represents a spliced combination of sequence obtained from an isolated clone

5 identified as "J422_fl", with additional 5' sequence obtained from a second double stranded clone. Clone J422 was isolated from a human activated peripheral blood mononuclear cell (PBMC) library using a trap which selects for nucleotides encoding secreted proteins; therefore, clone J422 does encode a secreted factor. J422 encodes a novel protein; BLASTN/BLASTX or FASTA searches revealed no exact sequence
10 matches. However, a FASTA search revealed homology between the J422 protein (in the approximate region of amino acids 34-156 of SEQ ID NO:4) and a number of *Drosophila* leucine-rich repeat (LRR) proteins. Analysis of the full-length J422 sequences revealed that the conserved EGF-domain found in a number of LRR family members was not present in J422. Based upon these homologies, J422 and these
15 homologous proteins are expected to share at least some activities.

The sequence of a polynucleotide encoding another protein of the present invention is set forth in SEQ ID NO:5, with the coding region extending from nucleotides 76 to 474. This polynucleotide has been identified as "clone L105". The amino acid sequence of the protein encoded by clone L105 is set forth in SEQ ID
20 NO:6. Clone L105 was deposited with the American Type Culture Collection on August 11, 1995 and given the accession number ATCC 69883. Clone L105 was isolated from a murine adult thymus library using a trap which selects for nucleotides encoding secreted proteins; therefore, clone L105 does encode a secreted factor. L105 encodes a novel protein; BLASTN/BLASTX or FASTA searches revealed no exact
25 sequence matches. However, a BLASTX search revealed homology between the L105 protein (particularly in the approximate region of amino acids 73-91 of SEQ ID NO:6), various monocyte and other chemoattractant proteins (including without limitation those assigned accession M577441, X71087, X72308, X14768 and M24545) and a chicken (*Gallus gallus*) cytokine (accession L34553). Based upon
30 these homologies, L105 and these homologous proteins are expected to share at least some activities.

The sequence of polynucleotides encoding another protein of the present invention is set forth in SEQ ID NO:7 and SEQ ID NO:9, with the coding regions extending from nucleotides 67 to 348 and nucleotides 75 to 356, respectively. These
35 polynucleotides have been identified as "clone H174-10" and "clone H174-43", respectively (collectively referred to herein as "H174"). The amino acid sequence of

5 the protein encoded by clones H174 is set forth in SEQ ID NO:8 and SEQ ID NO:10. Clone H174 was deposited with the American Type Culture Collection on August 11, 1995 and given the accession number ATCC 69882. Clones H174 were isolated from a human activated peripheral blood mononuclear cell (PBMC) library using a trap which selects for nucleotides encoding secreted proteins; therefore, H174 does encode
10 a secreted factor. H174 encodes a novel protein; BLASTN/BLASTX or FASTA searches revealed no exact sequence matches. However, a BLASTX search revealed homology between the H174 protein, human IP-10 (accession M33266) and murine CRG-2 (accession M86820) (species homologs). Based upon these homologies, H174 and these homologous proteins are expected to share at least some activities.

15 The sequence of a polynucleotide encoding another protein of the present invention is set forth in SEQ ID NO:11, with the coding region extending from nucleotides 86 to 544. This polynucleotide has been identified as "B18". The amino acid sequence of the protein encoded by clone B18 is set forth in SEQ ID NO:12. Clone B18 was deposited with the American Type Culture Collection on July 6, 1995
20 and assigned accession number ATCC 69868. Clone B18 was isolated from a human activated peripheral blood mononuclear cell (PBMC) library using a trap which selects for nucleotides encoding secreted proteins; therefore, clone B18 does encode a secreted factor. B18 encodes a novel protein; BLASTN/BLASTX or FASTA searches revealed no exact sequence matches. However, a BLASTX search revealed that the
25 region from amino acid 29 to amino acid 163 of B18 (SEQ ID NO:12) shows marked homology to portions of murine CTLA-8 (amino acids 18 to 150, accession L13839) and herpesvirus *Saimiri* ORF13 ("herpes CTLA-8") (amino acids 19 to 151, accession X64346). Based upon these homologies, B18 is believed to be the human homolog of murine and herpes CTLA-8 (i.e., "human CTLA-8"). B18 may demonstrate proinflammatory activity, particularly in development of T-cell dependent immune
30 responses. B18 is also expected to possess other activities specified herein.

Clones J5, L105, H174 and B18 were each transfected into COS cells labelled with ³⁵S-methionine and protein was expressed. Autoradiographs evidencing expression of the proteins in conditioned media are presented in Figs. 1, 2, 3 and 4,

5 respectively. The bands of protein expressed from the relevant clone are indicated by arrows.

Polynucleotides hybridizing to the polynucleotides of the present invention under stringent conditions and highly stringent conditions are also part of the present invention. As used herein, "highly stringent conditions" include, for example, at least about 10 0.2xSSC at 65°C; and "stringent conditions" include, for example, at least about 4xSSC at 65°C or at least about 50% formamide, 4xSSC at 42°C. Allelic variants of the polynucleotides of the present invention are also encompassed by the invention.

Fragments of the proteins of the present invention which are capable of exhibiting biological activity are also encompassed by the present invention. 15 Fragments of the protein may be in linear form or they may be cyclized using known methods, for example, as described in H.U. Saragovi, *et al.*, BioTechnology 10, 773-778 (1992) and in R.S. McDowell, *et al.*, J. Amer. Chem. Soc. 114, 9245-9253 (1992), both of which are incorporated herein by reference. Such fragments may be fused to carrier molecules such as immunoglobulins for many purposes, including increasing 20 the valency of protein binding sites. For example, fragments of the protein may be fused through "linker" sequences to the Fc portion of an immunoglobulin. For a bivalent form of the protein, such a fusion could be to the Fc portion of an IgG molecule. Other immunoglobulin isotypes may also be used to generate such fusions. For example, a protein - IgM fusion would generate a decavalent form of the protein 25 of the invention.

The isolated polynucleotide of the invention may be operably linked to an expression control sequence such as the pMT2 or pED expression vectors disclosed in Kaufman *et al.*, Nucleic Acids Res. 19, 4485-4490 (1991), in order to produce the protein recombinantly. Many suitable expression control sequences are known in the 30 art. General methods of expressing recombinant proteins are also known and are exemplified in R. Kaufman, Methods in Enzymology 185, 537-566 (1990). As defined herein "operably linked" means that the isolated polynucleotide of the invention and an expression control sequence are situated within a vector or cell in such a way that the protein is expressed by a host cell which has been transformed 35 (transfected) with the ligated polynucleotide/expression control sequence.

5 A number of types of cells may act as suitable host cells for expression of the protein. Mammalian host cells include, for example, monkey COS cells, Chinese Hamster Ovary (CHO) cells, human kidney 293 cells, human epidermal A431 cells, human Colo205 cells, 3T3 cells, CV-1 cells, other transformed primate cell lines, normal diploid cells, cell strains derived from in vitro culture of primary tissue, 10 primary explants, HeLa cells, mouse L cells, BHK, HL-60, U937, HaK or Jurkat cells.

15 Alternatively, it may be possible to produce the protein in lower eukaryotes such as yeast or in prokaryotes such as bacteria. Potentially suitable yeast strains include *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Kluyveromyces* strains, *Candida*, or any yeast strain capable of expressing heterologous proteins. Potentially suitable bacterial strains include *Escherichia coli*, *Bacillus subtilis*, *Salmonella typhimurium*, or any bacterial strain capable of expressing heterologous proteins. If the protein is made in yeast or bacteria, it may be necessary to modify the protein produced therein, for example by phosphorylation or glycosylation of the appropriate sites, in order to obtain the functional protein. Such covalent attachments 20 may be accomplished using known chemical or enzymatic methods.

25 The protein may also be produced by operably linking the isolated polynucleotide of the invention to suitable control sequences in one or more insect expression vectors, and employing an insect expression system. Materials and methods for baculovirus/insect cell expression systems are commercially available in kit form from, e.g., Invitrogen, San Diego, California, U.S.A. (the MaxBac® kit), and such methods are well known in the art, as described in Summers and Smith, Texas Agricultural Experiment Station Bulletin No. 1555 (1987), incorporated herein by reference. As used herein, an insect cell capable of expressing a polynucleotide of the present invention is "transformed."

30 The protein of the invention may be prepared by culturing transformed host cells under culture conditions suitable to express the recombinant protein. The resulting expressed protein may then be purified from such culture (i.e., from culture medium or cell extracts) using known purification processes, such as gel filtration and ion exchange chromatography. The purification of the protein may also include an 35 affinity column containing agents which will bind to the protein; one or more column steps over such affinity resins as concanavalin A-agarose, heparin-toyopearl® or

- 5 Cibacron blue 3GA Sepharose®; one or more steps involving hydrophobic interaction chromatography using such resins as phenyl ether, butyl ether, or propyl ether; or immunoaffinity chromatography.

10 Alternatively, the protein of the invention may also be expressed in a form which will facilitate purification. For example, it may be expressed as a fusion protein, such as those of maltose binding protein (MBP), glutathione-S-transferase (GST) or thioredoxin (TRX). Kits for expression and purification of such fusion proteins are commercially available from New England BioLab (Beverly, MA), Pharmacia (Piscataway, NJ) and InVitrogen, respectively. The protein can also be tagged with an epitope and subsequently purified by using a specific antibody directed 15 to such epitope. One such epitope ("Flag") is commercially available from Kodak (New Haven, CT).

20 Finally, one or more reverse-phase high performance liquid chromatography (RP-HPLC) steps employing hydrophobic RP-HPLC media, e.g., silica gel having pendant methyl or other aliphatic groups, can be employed to further purify the protein. Some or all of the foregoing purification steps, in various combinations, can also be employed to provide a substantially homogeneous isolated recombinant protein. The protein thus purified is substantially free of other mammalian proteins and is defined in accordance with the present invention as an "isolated protein."

25 The protein of the invention may also be expressed as a product of transgenic animals, e.g., as a component of the milk of transgenic cows, goats, pigs, or sheep which are characterized by somatic or germ cells containing a nucleotide sequence encoding the protein.

30 The protein may also be produced by known conventional chemical synthesis. Methods for constructing the proteins of the present invention by synthetic means are known to those skilled in the art. The synthetically-constructed protein sequences, by virtue of sharing primary, secondary or tertiary structural and/or conformational characteristics with proteins may possess biological properties in common therewith, including protein activity. Thus, they may be employed as biologically active or immunological substitutes for natural, purified proteins in screening of therapeutic 35 compounds and in immunological processes for the development of antibodies.

5 The proteins provided herein also include proteins characterized by amino acid sequences similar to those of purified proteins but into which modification are naturally provided or deliberately engineered. For example, modifications in the peptide or DNA sequences can be made by those skilled in the art using known techniques. Modifications of interest in the protein sequences may include the
10 replacement, insertion or deletion of a selected amino acid residue in the coding sequence. For example, one or more of the cysteine residues may be deleted or replaced with another amino acid to alter the conformation of the molecule. Mutagenic techniques for such replacement, insertion or deletion are well known to those skilled in the art (see, e.g., U.S. Patent No. 4,518,584).

15 Other fragments and derivatives of the sequences of proteins which would be expected to retain protein activity in whole or in part and may thus be useful for screening or other immunological methodologies may also be easily made by those skilled in the art given the disclosures herein. Such modifications are believed to be encompassed by the present invention.

20

USES AND BIOLOGICAL ACTIVITY

25 The polynucleotides of the present invention and the proteins encoded thereby are expected to exhibit one or more of the uses or biological activities (including those associated with assays cited herein) identified below. Uses or activities described for proteins of the present invention may be provided by administration or use of such proteins or by administration or use of polynucleotides encoding such proteins (such as, for example, in gene therapies or vectors suitable for introduction of DNA).

RESEARCH TOOL UTILITY

30 The polynucleotides provided by the present invention can be used by the research community for various purposes. The polynucleotides can be used to express recombinant protein for analysis, characterization or therapeutic use; as markers for tissues in which the corresponding protein is preferentially expressed (either constitutively or at a particular stage of tissue differentiation or development or in disease states); as molecular weight markers on Southern gels; as chromosome markers (when labeled) to map related gene positions; to compare with endogenous

5 DNA sequences in patients to identify potential genetic disorders; as probes to hybridize and thus discover novel, related DNA sequences; as a source of information to derive PCR primers for genetic fingerprinting; as a probe to "subtract-out" known sequences in the process of discovering other novel polynucleotides; to raise anti-protein antibodies using DNA immunization techniques; and as an antigen to raise
10 anti-DNA antibodies or elicit another immune response. Where the polynucleotide encodes a protein which binds or potentially binds to another protein (such as, for example, in a receptor-ligand interaction), the polynucleotide can also be used in interaction trap assays (such as, for example, that described in Gyuris et al., Cell 75:791-803 (1993)) to identify polynucleotides encoding the other protein with which
15 binding occurs or to identify inhibitors of the binding interaction.

The proteins provided by the present invention can similarly be used to raise antibodies or to elicit another immune response; as a reagent (including the labelled reagent) in assays designed to quantitatively determine levels of the protein (or its receptor) in biological fluids; as markers for tissues in which the corresponding
20 protein is preferentially expressed (either constitutively or at a particular stage of tissue differentiation or development or in a disease state); and, of course, to isolate correlative receptors or ligands. Where the protein binds or potentially binds to another protein (such as, for example, in a receptor-ligand interaction), the protein can be used to identify the other protein with which binding occurs or to identify inhibitors
25 of the binding interaction. Proteins involved in these binding interactions can also be used to screen for peptide or small molecule inhibitors or agonists of the binding interaction.

Any or all of these "research tool" utilities are capable of being developed into reagent grade or kit format for commercialization as "research products."

30

CYTOKINE AND CELL PROLIFERATION/DIFFERENTIATION ACTIVITY

A protein of the present invention may exhibit cytokine, cell proliferation (either inducing or inhibiting) or cell differentiation (either inducing or inhibiting)
35 activity or may induce production of other cytokines in certain cell populations. Many protein factors discovered to date, including all known cytokines, have exhibited

5 activity in one or more factor dependent cell proliferation assays, and hence the assays serve as a convenient confirmation of cytokine activity. The activity of a protein of the present invention is evidenced by any one of a number of routine factor dependent cell proliferation assays for cell lines including, without limitation, 32D, DA2, DA1G, T10, B9, B9/11, BaF3, MC9/G, M+ (preB M+), 2E8, RB5, DA1, 123, T1165, HT2, 10 CTLL2, TF-1, Mo7e and CMK.

The activity of a protein of the invention may, among other means, be measured by the following methods:

15 Assays for T-cell or thymocyte proliferation include without limitation those described in: Current Protocols in Immunology, Ed by J. E. Coligan, A.M. Kruisbeek, D.H. Margulies, E.M. Shevach, W Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 3, In Vitro assays for Mouse Lymphocyte Function 3.1-3.19; Chapter 7, Immunologic studies in Humans); Takai et al., J. Immunol. 137:3494-3500, 1986; Bertagnolli et al., J. Immunol. 145:1706-1712, 1990; 20 Bertagnolli et al., Cellular Immunology 133:327-341, 1991; Bertagnolli, et al., J. Immunol. 149:3778-3783, 1992; Bowman et al., J. Immunol. 152: 1756-1761, 1994.

Assays for cytokine production and/or proliferation of spleen cells, lymph node cells or thymocytes include, without limitation, those described in: Polyclonal T cell stimulation, Kruisbeek, A.M. and Shevach, E.M. In *Current Protocols in Immunology*. J.E.e.a. Coligan eds. Vol 1 pp. 3.12.1-3.12.14, John Wiley and Sons, 25 Toronto. 1994; and Measurement of mouse and human Interferon γ , Schreiber, R.D. In *Current Protocols in Immunology*. J.E.e.a. Coligan eds. Vol 1 pp. 6.8.1-6.8.8, John Wiley and Sons, Toronto. 1994.

30 Assays for proliferation and differentiation of hematopoietic and lymphopoietic cells include, without limitation, those described in: Measurement of Human and Murine Interleukin 2 and Interleukin 4, Bottomly, K., Davis, L.S. and Lipsky, P.E. In *Current Protocols in Immunology*. J.E.e.a. Coligan eds. Vol 1 pp. 6.3.1-6.3.12, John Wiley and Sons, Toronto. 1991; deVries et al., J. Exp. Med. 173:1205-1211, 1991; Moreau et al., Nature 336:690-692, 1988; Greenberger et al., Proc. Natl. Acad. Sci. U.S.A. 80:2931-2938, 1983; Measurement of mouse and human 35 interleukin 6 - Nordan, R. In *Current Protocols in Immunology*. J.E.e.a. Coligan eds. Vol 1 pp. 6.6.1-6.6.5, John Wiley and Sons, Toronto. 1991; Smith et al., Proc. Natl.

5 Acad. Sci. U.S.A. 83:1857-1861, 1986; Measurement of human Interleukin 11 - Bennett, F., Giannotti, J., Clark, S.C. and Turner, K. J. In *Current Protocols in Immunology*. J.E.e.a. Coligan eds. Vol 1 pp. 6.15.1 John Wiley and Sons, Toronto. 1991; Measurement of mouse and human Interleukin 9 - Ciarletta, A., Giannotti, J., Clark, S.C. and Turner, K.J. In *Current Protocols in Immunology*. J.E.e.a. Coligan eds. Vol 1 pp. 6.13.1, John Wiley and Sons, Toronto. 1991.

10 15 Assays for T-cell clone responses to antigens (which will identify, among others, proteins that affect APC-T cell interactions as well as direct T-cell effects by measuring proliferation and cytokine production) include, without limitation, those described in: Current Protocols in Immunology, Ed by J. E. Coligan, A.M. Kruisbeek, D.H. Margulies, E.M. Shevach, W Strober

Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 3, In Vitro assays for Mouse Lymphocyte Function; Chapter 6, Cytokines and their cellular receptors; Chapter 7, Immunologic studies in Humans); Weinberger et al., Proc. Natl. Acad. Sci. USA 77:6091-6095, 1980; Weinberger et al., Eur. J. Immun. 11:405-411, 1981; Takai et al., J. Immunol. 137:3494-3500, 1986; Takai et al., J. Immunol. 140:508-512, 1988.

IMMUNE STIMULATING/SUPPRESSING ACTIVITY

20 25 30 35 A protein of the present invention may also exhibit immune stimulating or immune suppressing activity, including without limitation the activities for which assays are described herein. A protein may be useful in the treatment of various immune deficiencies and disorders (including severe combined immunodeficiency (SCID)), e.g., in regulating (up or down) growth and proliferation of T and/or B lymphocytes, as well as effecting the cytolytic activity of NK cells and other cell populations. These immune deficiencies may be genetic or be caused by viral (e.g., HIV) as well as bacterial or fungal infections, or may result from autoimmune disorders. More specifically, infectious diseases causes by viral, bacterial, fungal or other infection may be treatable using a protein of the present invention, including infections by HIV, hepatitis viruses, herpes viruses, mycobacteria, leshmania, malaria and various fungal infections such as candida. Of course, in this regard, a protein of

5 the present invention may also be useful where a boost to the immune system generally would be indicated, i.e., in the treatment of cancer.

10 Autoimmune disorders which may be treated using a protein of the present invention include, for example, multiple sclerosis, systemic lupus erythematosus, rheumatoid arthritis, autoimmune pulmonary inflammation, Guillain-Barre syndrome, 15 autoimmune thyroiditis, insulin dependent diabetes mellitus, myasthenia gravis, graft-versus-host disease and autoimmune inflammatory eye disease. Such a protein of the present invention may also be useful in the treatment of allergic reactions and conditions, such as asthma or other respiratory problems. Other conditions, in which immune suppression is desired (including, for example, asthma and related respiratory 20 conditions), may also be treatable using a protein of the present invention.

A protein of the present invention may also suppress chronic or acute inflammation, such as, for example, that associated with infection (such as septic shock or systemic inflammatory response syndrome (SIRS)), inflammatory bowel disease, Crohn's disease or resulting from over production of cytokines such as TNF 25 or IL-1 (such as the effect demonstrated by IL-11).

The activity of a protein of the invention may, among other means, be measured by the following methods:

Suitable assays for thymocyte or splenocyte cytotoxicity include, without limitation, those described in: Current Protocols in Immunology, Ed by J. E. Coligan, 25 A.M. Kruisbeek, D.H. Margulies, E.M. Shevach, W Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 3, In Vitro assays for Mouse Lymphocyte Function 3.1-3.19; Chapter 7, Immunologic studies in Humans); Herrmann et al., Proc. Natl. Acad. Sci. USA 78:2488-2492, 1981; Herrmann et al., J. Immunol. 128:1968-1974, 1982; Handa et al., J. Immunol. 135:1564-1572, 1985; Takai et al., 30 J. Immunol. 137:3494-3500, 1986; Takai et al., J. Immunol. 140:508-512, 1988; Herrmann et al., Proc. Natl. Acad. Sci. USA 78:2488-2492, 1981; Herrmann et al., J. Immunol. 128:1968-1974, 1982; Handa et al., J. Immunol. 135:1564-1572, 1985; Takai et al., J. Immunol. 137:3494-3500, 1986; Bowman et al., J. Virology 61:1992-1998; Takai et al., J. Immunol. 140:508-512, 1988; Bertagnolli et al., Cellular 35 Immunology 133:327-341, 1991; Brown et al., J. Immunol. 153:3079-3092, 1994.

5 Assays for T-cell-dependent immunoglobulin responses and isotype switching
which will identify, among others, proteins that modulate T-cell dependent antibody
responses and that affect Th1/Th2 profiles) include, without limitation, those
described in: Maliszewski, J. Immunol. 144:3028-3033, 1990; and Assays for B cell
10 function: *In vitro* antibody production, Mond, J.J. and Brunswick, M. In *Current
Protocols in Immunology*. J.E.e.a. Coligan eds. Vol 1 pp. 3.8.1-3.8.16, John Wiley and
Sons, Toronto. 1994.

15 Mixed lymphocyte reaction (MLR) assays (which will identify, among others,
proteins that generate predominantly Th1 and CTL responses) include, without
limitation, those described in: Current Protocols in Immunology, Ed by J. E. Coligan,
A.M. Kruisbeek, D.H. Margulies, E.M. Shevach, W Strober, Pub. Greene Publishing
Associates and Wiley-Interscience (Chapter 3, In Vitro assays for Mouse Lymphocyte
Function 3.1-3.19; Chapter 7, Immunologic studies in Humans); Takai et al., J.
Immunol. 137:3494-3500, 1986; Takai et al.; J. Immunol. 140:508-512, 1988;
Bertagnolli et al., J. Immunol. 149:3778-3783, 1992.

20 Dendritic cell-dependent assays (which will identify, among others, proteins
expressed by denritic cells that activate naive T-cells) include, without limitation,
those described in: Guery et al., J. Immunol. 134:536-544, 1995; Inaba et al., Journal
of Experimental Medicine 173:549-559, 1991; Macatonia et al., Journal of
Immunology 154:5071-5079, 1995; Porgador et al., Journal of Experimental Medicine
25 182:255-260, 1995; Nair et al., Journal of Virology 67:4062-4069, 1993; Huang et
al., Science 264:961-965, 1994; Macatonia et al., Journal of Experimental Medicine
169:1255-1264, 1989; Bhardwaj et al., Journal of Clinical Investigation 94:797-807,
1994; and Inaba et al., Journal of Experimental Medicine 172:631-640, 1990.

30 Assays for lymphocyte survival/apoptosis (which will identify, among others,
proteins that prevent apoptosis after superantigen induction and proteins that regulate
lymphocyte homeostasis) include, without limitation, those described in:
Darzynkiewicz et al., Cytometry 13:795-808, 1992; Gorczyca et al., Leukemia
7:659-670, 1993; Gorczyca et al., Cancer Research 53:1945-1951, 1993; Itoh et al.,
Cell 66:233-243, 1991; Zacharchuk, Journal of Immunology 145:4037-4045, 1990;
35 Zamai et al., Cytometry 14:891-897, 1993; Gorczyca et al., International Journal of
Oncology 1:639-648, 1992.

5 Assays for proteins that influence early steps of T-cell commitment and development include, without limitation, those described in: Antica et al., Blood 84:111-117, 1994; Fine et al., Cellular Immunology 155:111-122, 1994; Galy et al., Blood 85:2770-2778, 1995; Toki et al., Proc. Nat. Acad Sci. USA 88:7548-7551, 1991.

10

HEMATOPOIESIS REGULATING ACTIVITY

A protein of the present invention may be useful in regulation of hematopoiesis and, consequently, in the treatment of myeloid or lymphoid cell deficiencies. Even marginal biological activity in support of colony forming cells or of factor-dependent cell lines indicates involvement in regulating hematopoiesis. e.g. in supporting the growth and proliferation of erythroid progenitor cells alone or in combination with other cytokines, thereby indicating utility, for example, in treating various anemias or for use in conjunction with irradiation/chemotherapy to stimulate the production of erythroid precursors and/or erythroid cells; in supporting the growth and proliferation of myeloid cells such as granulocytes and monocytes/macrophages (i.e., traditional CSF activity) useful, for example, in conjunction with chemotherapy to prevent or treat consequent myelo-suppression; in supporting the growth and proliferation of megakaryocytes and consequently of platelets thereby allowing prevention or treatment of various platelet disorders such as thrombocytopenia, and generally for use in place of or complimentarily to platelet transfusions; and/or in supporting the growth and proliferation of hematopoietic stem cells which are capable of maturing to any and all of the above-mentioned hematopoietic cells and therefore find therapeutic utility in various stem cell disorders (such as those usually treated with transplantation, including, without limitation, aplastic anemia and paroxysmal nocturnal hemoglobinuria), as well as in repopulating the stem cell compartment post irradiation/chemotherapy, either *in-vivo* or *ex-vivo* (i.e. in conjunction with bone marrow transplantation) as normal cells or genetically manipulated for gene therapy.

The activity of a protein of the invention may, among other means, be measured by the following methods:

35 Suitable assays for proliferation and differentiation of various hematopoietic lines are cited above.

- 5 Assays for embryonic stem cell differentiation (which will identify, among others, proteins that influence embryonic differentiation hematopoiesis) include, without limitation, those described in: Johansson et al. *Cellular Biology* 15:141-151, 1995; Keller et al., *Molecular and Cellular Biology* 13:473-486, 1993; McClanahan et al., *Blood* 81:2903-2915, 1993.
- 10 Assays for stem cell survival and differentiation (which will identify, among others, proteins that regulate lympho-hematopoiesis) include, without limitation, those described in: Methylcellulose colony forming assays, Freshney, M.G. In *Culture of Hematopoietic Cells*. R.I. Freshney, *et al.* eds. Vol pp. 265-268, Wiley-Liss, Inc., New York, NY. 1994; Hirayama et al., *Proc. Natl. Acad. Sci. USA* 89:5907-5911, 1992; Primitive hematopoietic colony forming cells with high proliferative potential, McNiece, I.K. and Briddell, R.A. In *Culture of Hematopoietic Cells*. R.I. Freshney, *et al.* eds. Vol pp. 23-39, Wiley-Liss, Inc., New York, NY. 1994; Neben et al., *Experimental Hematology* 22:353-359, 1994; Cobblestone area forming cell assay, Ploemacher, R.E. In *Culture of Hematopoietic Cells*. R.I. Freshney, *et al.* eds. Vol pp. 1-21, Wiley-Liss, Inc., New York, NY. 1994; Long term bone marrow cultures in the presence of stromal cells, Spooncer, E., Dexter, M. and Allen, T. In *Culture of Hematopoietic Cells*. R.I. Freshney, *et al.* eds. Vol pp. 163-179, Wiley-Liss, Inc., New York, NY. 1994; Long term culture initiating cell assay, Sutherland, H.J. In *Culture of Hematopoietic Cells*. R.I. Freshney, *et al.* eds. Vol pp. 139-162, Wiley-Liss, Inc., New York, NY. 1994.

TISSUE GENERATION/REGENERATION ACTIVITY

A protein of the present invention also may have utility in compositions used for bone, cartilage, tendon, ligament and/or nerve tissue growth or regeneration, as well as for wound healing and tissue repair, and in the treatment of burns, incisions and ulcers.

A protein of the present invention, which induces cartilage and/or bone growth in circumstances where bone is not normally formed, has application in the healing of bone fractures and cartilage damage or defects in humans and other animals. Such a preparation employing a protein of the invention may have prophylactic use in closed as well as open fracture reduction and also in the improved fixation of artificial joints.

5 *De novo* bone formation induced by an osteogenic agent contributes to the repair of congenital, trauma induced, or oncologic resection induced craniofacial defects, and also is useful in cosmetic plastic surgery.

A protein of this invention may also be used in the treatment of periodontal disease, and in other tooth repair processes. Such agents may provide an environment 10 to attract bone-forming cells, stimulate growth of bone-forming cells or induce differentiation of progenitors of bone-forming cells. A protein of the invention may also be useful in the treatment of osteoporosis or osteoarthritis, such as through stimulation of bone and/or cartilage repair or by blocking inflammation or processes 15 of tissue destruction (collagenase activity, osteoclast activity, etc.) mediated by inflammatory processes.

Another category of tissue regeneration activity that may be attributable to the protein of the present invention is tendon/ligament formation. A protein of the present invention, which induces tendon/ligament-like tissue or other tissue formation in 20 circumstances where such tissue is not normally formed, has application in the healing of tendon or ligament tears, deformities and other tendon or ligament defects in humans and other animals. Such a preparation employing a tendon/ligament-like tissue inducing protein may have prophylactic use in preventing damage to tendon or ligament tissue, as well as use in the improved fixation of tendon or ligament to bone 25 or other tissues, and in repairing defects to tendon or ligament tissue. *De novo* tendon/ligament-like tissue formation induced by a composition of the present invention contributes to the repair of congenital, trauma induced, or other tendon or ligament defects of other origin, and is also useful in cosmetic plastic surgery for attachment or repair of tendons or ligaments. The compositions of the present 30 invention may provide an environment to attract tendon- or ligament-forming cells, stimulate growth of tendon- or ligament-forming cells, induce differentiation of progenitors of tendon- or ligament-forming cells, or induce growth of tendon/ligament cells or progenitors *ex vivo* for return *in vivo* to effect tissue repair. The compositions of the invention may also be useful in the treatment of tendinitis, carpal tunnel syndrome and other tendon or ligament defects. The compositions may also include 35 an appropriate matrix and/or sequestering agent as a carrier as is well known in the art.

5 The protein of the present invention may also be useful for proliferation of neural cells and for regeneration of nerve and brain tissue, *i.e.* for the treatment of central and peripheral nervous system diseases and neuropathies, as well as mechanical and traumatic disorders, which involve degeneration, death or trauma to neural cells or nerve tissue. More specifically, a protein may be used in the treatment
10 of diseases of the peripheral nervous system, such as peripheral nerve injuries, peripheral neuropathy and localized neuropathies, and central nervous system diseases, such as Alzheimer's, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, and Shy-Drager syndrome. Further conditions which may be treated in accordance with the present invention include mechanical and traumatic disorders,
15 such as spinal cord disorders, head trauma and cerebrovascular diseases such as stroke. Peripheral neuropathies resulting from chemotherapy or other medical therapies may also be treatable using a protein of the invention.

It is expected that a protein of the present invention may also exhibit activity for generation of other tissues, such as organs (including, for example, pancreas, liver, intestine, kidney, skin, endothelium), muscle (smooth, skeletal or cardiac) and vascular (including vascular endothelium) tissue, or for promoting the growth of cells comprising such tissues. Part of the desired effects may be by inhibition of fibrotic scarring to allow normal tissue to regenerate.

25 A protein of the present invention may also be useful for gut protection or regeneration and treatment of lung or liver fibrosis, reperfusion injury in various tissues, and conditions resulting from systemic cytokine damage.

The activity of a protein of the invention may, among other means, be measured by the following methods:

30 Assays for tissue generation activity include, without limitation, those described in: International Patent Publication No. WO95/16035 (bone, cartilage, tendon); International Patent Publication No. WO95/05846 (nerve, neuronal); International Patent Publication No. WO91/07491 (skin, endothelium).

ACTIVIN/INHIBIN ACTIVITY

35 A protein of the present invention may also exhibit activin- or inhibin-related activities. Inhibins are characterized by their ability to inhibit the release of follicle

5 stimulating hormone (FSH), while activins and are characterized by their ability to stimulate the release of follicle stimulating hormone (FSH). Thus, a protein of the present invention, alone or in heterodimers with a member of the inhibin α family, may be useful as a contraceptive based on the ability of inhibins to decrease fertility in female mammals and decrease spermatogenesis in male mammals. Administration
10 of sufficient amounts of other inhibins can induce infertility in these mammals. Alternatively, the protein of the invention, as a homodimer or as a heterodimer with other protein subunits of the inhibin- β group, may be useful as a fertility inducing therapeutic, based upon the ability of activin molecules in stimulating FSH release from cells of the anterior pituitary. See, for example, United States Patent 4,798,885.
15 A protein of the invention may also be useful for advancement of the onset of fertility in sexually immature mammals, so as to increase the lifetime reproductive performance of domestic animals such as cows, sheep and pigs.

The activity of a protein of the invention may, among other means, be measured by the following methods:

20 Assays for activin/inhibin activity include, without limitation, those described in: Vale et al., Endocrinology 91:562-572, 1972; Ling et al., Nature 321:779-782, 1986; Vale et al., Nature 321:776-779, 1986; Mason et al., Nature 318:659-663, 1985; Forage et al., Proc. Natl. Acad. Sci. USA 83:3091-3095, 1986.

25

CHEMOTACTIC/CHEMOKINETIC ACTIVITY

A protein of the present invention may have chemotactic or chemokinetic activity (e.g., act as a chemokine) for mammalian cells, including, for example, monocytes, neutrophils, T-cells, mast cells, eosinophils and/or endothelial cells. Chemotactic and chemokinetic proteins can be used to mobilized or attract a desired cell population to a desired site of action. Chemotactic or chemokinetic proteins provide particular advantages in treatment of wounds and other trauma to tissues, as well as in treatment of localized infections. For example, attraction of lymphocytes, monocytes or neutrophils to tumors or sites of infection may result in improved immune responses against the tumor or infecting agent.

35

A protein or peptide has chemotactic activity for a particular cell population if it can stimulate, directly or indirectly, the directed orientation or movement of such

5 cell population. Preferably, the protein or peptide has the ability to directly stimulate directed movement of cells. Whether a particular protein has chemotactic activity for a population of cells can be readily determined by employing such protein or peptide in any known assay for cell chemotaxis.

10 The activity of a protein of the invention may, among other means, be measured by the following methods:

15 Assays for chemotactic activity (which will identify proteins that induce or prevent chemotaxis) consist of assays that measure the ability of a protein to induce the migration of cells across a membrane as well as the ability of a protein to induce the adhesion of one cell population to another cell population. Suitable assays for movement and adhesion include, without limitation, those described in: Current Protocols in Immunology, Ed by J.E. Coligan, A.M. Kruisbeek, D.H. Margulies, E.M. Shevach, W. Strober, Pub. Greene Publishing Associates and Wiley-Intersciece (Chapter 6.12, Measurement of alpha and beta Chemokines 6.12.1-6.12.28; Taub et al. J. Clin. Invest. 95:1370-1376, 1995; Lind et al. APMIS 103:140-146, 1995; Muller 20 et al Eur. J. Immunol. 25: 1744-1748; Gruber et al. J. of Immunol. 152:5860-5867, 1994; Johnston et al. J. of Immunol. 153: 1762-1768, 1994.

HEMOSTATIC AND THROMBOLYTIC ACTIVITY

25 A protein of the invention may also exhibit hemostatic or thrombolytic activity. As a result, such a protein is expected to be useful in treatment of various coagulation disorders (including hereditary disorders, such as hemophilias) or to enhance coagulation and other hemostatic events in treating wounds resulting from trauma, surgery or other causes. A protein of the invention may also be useful for dissolving or inhibiting formation of thromboses and for treatment and prevention of 30 conditions resulting therefrom (such as, for example, infarction or stroke).

 The activity of a protein of the invention may, among other means, be measured by the following methods:

35 Assay for hemostatic and thrombolytic activity include, without limitation, those described in: Linet et al., J. Clin. Pharmacol. 26:131-140, 1986; Burdick et al., Thrombosis Res. 45:413-419, 1987; Humphrey et al., Fibrinolysis 5:71-79 (1991); Schaub, Prostaglandins 35:467-474, 1988.

5

RECEPTOR/LIGAND ACTIVITY

A protein of the present invention may also demonstrate activity as receptors, receptor ligands or inhibitors or agonists of receptor/ligand interactions. Examples of such receptors and ligands include, without limitation, cytokine receptors and their ligands, receptor kinases and their ligands, receptor phosphatases and their ligands, 10 receptors involved in cell-cell interactions and their ligands (including without limitation, cellular adhesion molecules (such as selectins, integrins and their ligands) and receptor/ligand pairs involved in antigen presentation, antigen recognition and development of cellular and humoral immune responses). Receptors and ligands are also useful for screening of potential peptide or small molecule inhibitors of the 15 relevant receptor/ligand interaction. A protein of the present invention (including, without limitation, fragments of receptors and ligands) may themselves be useful as inhibitors of receptor/ligand interactions.

The activity of a protein of the invention may, among other means, be measured by the following methods:

20

Suitable assays for receptor-ligand activity include without limitation those described in: Current Protocols in Immunology, Ed by J.E. Coligan, A.M. Kruisbeek, D.H. Margulies, E.M. Shevach, W. Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 7.28, Measurement of Cellular Adhesion under static conditions 7.28.1-7.28.22), Takai et al., Proc. Natl. Acad. Sci. USA 84:6864-6868, 25 1987; Bierer et al., J. Exp. Med. 168:1145-1156, 1988; Rosenstein et al., J. Exp. Med. 169:149-160 1989; Stoltenborg et al., J. Immunol. Methods 175:59-68, 1994; Stitt et al., Cell 80:661-670, 1995.

OTHER ACTIVITIES

30

A protein of the invention may also exhibit one or more of the following additional activities or effects: killing infectious agents, including, without limitation, bacteria, viruses, fungi and other parasites; effecting (suppressing or enhancing) bodily characteristics, including, without limitation, height, weight, hair color, eye color, skin or other tissue pigmentation, or organ size (such as, for example, breast augmentation or diminution); effecting the processing of dietary fat, protein or carbohydrate; effecting behavioral characteristics, including, without limitation, appetite, libido,

5 stress, cognition (including cognitive disorders), depression (including depressive disorders) and violent behaviors; providing analgesic effects or other pain reducing effects; promoting differentiation and growth of embryonic stem cells in lineages other than hematopoietic lineages; and in the case of enzymes, correcting deficiencies of the enzyme and treating related diseases.

10

ADMINISTRATION AND DOSING

A protein of the present invention (from whatever source derived, including without limitation from recombinant and non-recombinant sources) may be used in a pharmaceutical composition when combined with a pharmaceutically acceptable carrier. Such a composition may also contain (in addition to protein and a carrier) diluents, fillers, salts, buffers, stabilizers, solubilizers, and other materials well known in the art. The term "pharmaceutically acceptable" means a non-toxic material that does not interfere with the effectiveness of the biological activity of the active ingredient(s). The characteristics of the carrier will depend on the route of administration. The pharmaceutical composition of the invention may also contain cytokines, lymphokines, or other hematopoietic factors such as M-CSF, GM-CSF, TNF, IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IFN, TNF₀, TNF₁, TNF₂, G-CSF, Meg-CSF, thrombopoietin, stem cell factor, and erythropoietin. The pharmaceutical composition may further contain other agents which either enhance the activity of the protein or compliment its activity or use in treatment. Such additional factors and/or agents may be included in the pharmaceutical composition to produce a synergistic effect with protein of the invention, or to minimize side effects. Conversely, protein of the present invention may be included in formulations of the particular cytokine, lymphokine, other hematopoietic factor, thrombolytic or anti-thrombotic factor, or anti-inflammatory agent to minimize side effects of the cytokine, lymphokine, other hematopoietic factor, thrombolytic or anti-thrombotic factor, or anti-inflammatory agent.

A protein of the present invention may be active in multimers (e.g., heterodimers or homodimers) or complexes with itself or other proteins. As a result, pharmaceutical compositions of the invention may comprise a protein of the invention in such multimeric or complexed form.

5 The pharmaceutical composition of the invention may be in the form of a complex of the protein(s) of present invention along with protein or peptide antigens. The protein and/or peptide antigen will deliver a stimulatory signal to both B and T lymphocytes. B lymphocytes will respond to antigen through their surface immunoglobulin receptor. T lymphocytes will respond to antigen through the T cell receptor (TCR) following presentation of the antigen by MHC proteins. MHC and structurally related proteins including those encoded by class I and class II MHC genes on host cells will serve to present the peptide antigen(s) to T lymphocytes. The antigen components could also be supplied as purified MHC-peptide complexes alone or with co-stimulatory molecules that can directly signal T cells. Alternatively
10 antibodies able to bind surface immunoglobulin and other molecules on B cells as well as antibodies able to bind the TCR and other molecules on T cells can be combined with the pharmaceutical composition of the invention.
15

The pharmaceutical composition of the invention may be in the form of a liposome in which protein of the present invention is combined, in addition to other pharmaceutically acceptable carriers, with amphipathic agents such as lipids which exist in aggregated form as micelles, insoluble monolayers, liquid crystals, or lamellar layers in aqueous solution. Suitable lipids for liposomal formulation include, without limitation, monoglycerides, diglycerides, sulfatides, lysolecithin, phospholipids, saponin, bile acids, and the like. Preparation of such liposomal formulations is within the level of skill in the art, as disclosed, for example, in U.S. Patent No. 4,235,871; U.S. Patent No. 4,501,728; U.S. Patent No. 4,837,028; and U.S. Patent No. 4,737,323, all of which are incorporated herein by reference.

As used herein, the term "therapeutically effective amount" means the total amount of each active component of the pharmaceutical composition or method that is sufficient to show a meaningful patient benefit, i.e., treatment, healing, prevention or amelioration of the relevant medical condition, or an increase in rate of treatment, healing, prevention or amelioration of such conditions. When applied to an individual active ingredient, administered alone, the term refers to that ingredient alone. When applied to a combination, the term refers to combined amounts of the active ingredients that result in the therapeutic effect, whether administered in combination, serially or simultaneously.

5 In practicing the method of treatment or use of the present invention, a therapeutically effective amount of protein of the present invention is administered to a mammal having a condition to be treated. Protein of the present invention may be administered in accordance with the method of the invention either alone or in combination with other therapies such as treatments employing cytokines,
10 lymphokines or other hematopoietic factors. When co-administered with one or more cytokines, lymphokines or other hematopoietic factors, protein of the present invention may be administered either simultaneously with the cytokine(s), lymphokine(s), other hematopoietic factor(s), thrombolytic or anti-thrombotic factors, or sequentially. If administered sequentially, the attending physician will decide on
15 the appropriate sequence of administering protein of the present invention in combination with cytokine(s), lymphokine(s), other hematopoietic factor(s), thrombolytic or anti-thrombotic factors.

20 Administration of protein of the present invention used in the pharmaceutical composition or to practice the method of the present invention can be carried out in a variety of conventional ways, such as oral ingestion, inhalation, or cutaneous, subcutaneous, or intravenous injection. Intravenous administration to the patient is preferred.

25 When a therapeutically effective amount of protein of the present invention is administered orally, protein of the present invention will be in the form of a tablet, capsule, powder, solution or elixir. When administered in tablet form, the pharmaceutical composition of the invention may additionally contain a solid carrier such as a gelatin or an adjuvant. The tablet, capsule, and powder contain from about 5 to 95% protein of the present invention, and preferably from about 25 to 90% protein of the present invention. When administered in liquid form, a liquid carrier such as water, petroleum, oils of animal or plant origin such as peanut oil, mineral oil, soybean oil, or sesame oil, or synthetic oils may be added. The liquid form of the pharmaceutical composition may further contain physiological saline solution, dextrose or other saccharide solution, or glycols such as ethylene glycol, propylene glycol or polyethylene glycol. When administered in liquid form, the pharmaceutical composition contains from about 0.5 to 90% by weight of protein of the present invention, and preferably from about 1 to 50% protein of the present invention.
30
35

5 When a therapeutically effective amount of protein of the present invention is administered by intravenous, cutaneous or subcutaneous injection, protein of the present invention will be in the form of a pyrogen-free, parenterally acceptable aqueous solution. The preparation of such parenterally acceptable protein solutions, having due regard to pH, isotonicity, stability, and the like, is within the skill in the art.

10 A preferred pharmaceutical composition for intravenous, cutaneous, or subcutaneous injection should contain, in addition to protein of the present invention, an isotonic vehicle such as Sodium Chloride Injection, Ringer's Injection, Dextrose Injection, Dextrose and Sodium Chloride Injection, Lactated Ringer's Injection, or other vehicle as known in the art. The pharmaceutical composition of the present invention may

15 also contain stabilizers, preservatives, buffers, antioxidants, or other additives known to those of skill in the art.

20 The amount of protein of the present invention in the pharmaceutical composition of the present invention will depend upon the nature and severity of the condition being treated, and on the nature of prior treatments which the patient has undergone. Ultimately, the attending physician will decide the amount of protein of the present invention with which to treat each individual patient. Initially, the attending physician will administer low doses of protein of the present invention and observe the patient's response. Larger doses of protein of the present invention may be administered until the optimal therapeutic effect is obtained for the patient, and at

25 that point the dosage is not increased further. It is contemplated that the various pharmaceutical compositions used to practice the method of the present invention should contain about 0.01 µg to about 100 mg (preferably about 0.1µg to about 10 mg, more preferably about 0.1 µg to about 1 mg) of protein of the present invention per kg body weight.

30 The duration of intravenous therapy using the pharmaceutical composition of the present invention will vary, depending on the severity of the disease being treated and the condition and potential idiosyncratic response of each individual patient. It is contemplated that the duration of each application of the protein of the present invention will be in the range of 12 to 24 hours of continuous intravenous administration. Ultimately the attending physician will decide on the appropriate

5 duration of intravenous therapy using the pharmaceutical composition of the present invention.

Protein of the invention may also be used to immunize animals to obtain polyclonal and monoclonal antibodies which specifically react with the protein. Such antibodies may be obtained using either the entire protein or fragments thereof as an 10 immunogen. The peptide immunogens additionally may contain a cysteine residue at the carboxyl terminus, and are conjugated to a hapten such as keyhole limpet hemocyanin (KLH). Methods for synthesizing such peptides are known in the art, for example, as in R.P. Merrifield, J. Amer.Chem.Soc. 85, 2149-2154 (1963); J.L. Krstenansky, *et al.*, FEBS Lett. 211, 10 (1987). Monoclonal antibodies binding to the 15 protein of the invention may be useful diagnostic agents for the immunodetection of the protein. Neutralizing monoclonal antibodies binding to the protein may also be useful therapeutics for both conditions associated with the protein and also in the treatment of some forms of cancer where abnormal expression of the protein is involved. In the case of cancerous cells or leukemic cells, neutralizing monoclonal 20 antibodies against the protein may be useful in detecting and preventing the metastatic spread of the cancerous cells, which may be mediated by the protein.

For compositions of the present invention which are useful for bone, cartilage, tendon or ligament regeneration, the therapeutic method includes administering the 25 composition topically, systematically, or locally as an implant or device. When administered, the therapeutic composition for use in this invention is, of course, in a pyrogen-free, physiologically acceptable form. Further, the composition may desirably be encapsulated or injected in a viscous form for delivery to the site of bone, cartilage or tissue damage. Topical administration may be suitable for wound healing and tissue repair. Therapeutically useful agents other than a protein of the invention 30 which may also optionally be included in the composition as described above, may alternatively or additionally, be administered simultaneously or sequentially with the composition in the methods of the invention. Preferably for bone and/or cartilage formation, the composition would include a matrix capable of delivering the protein-containing composition to the site of bone and/or cartilage damage, providing a 35 structure for the developing bone and cartilage and optimally capable of being

5 resorbed into the body. Such matrices may be formed of materials presently in use for other implanted medical applications.

The choice of matrix material is based on biocompatibility, biodegradability, mechanical properties, cosmetic appearance and interface properties. The particular application of the compositions will define the appropriate formulation. Potential
10 matrices for the compositions may be biodegradable and chemically defined calcium sulfate, tricalciumphosphate, hydroxyapatite, polylactic acid, polyglycolic acid and polyanhydrides. Other potential materials are biodegradable and biologically well-defined, such as bone or dermal collagen. Further matrices are comprised of pure proteins or extracellular matrix components. Other potential matrices are
15 nonbiodegradable and chemically defined, such as sintered hydroxapatite, bioglass, aluminates, or other ceramics. Matrices may be comprised of combinations of any of the above mentioned types of material, such as polylactic acid and hydroxyapatite or collagen and tricalciumphosphate. The bioceramics may be altered in composition, such as in calcium-aluminate-phosphate and processing to alter pore size, particle size,
20 particle shape, and biodegradability.

Presently preferred is a 50:50 (mole weight) copolymer of lactic acid and glycolic acid in the form of porous particles having diameters ranging from 150 to 800 microns. In some applications, it will be useful to utilize a sequestering agent, such as carboxymethyl cellulose or autologous blood clot, to prevent the protein
25 compositions from disassociating from the matrix.

A preferred family of sequestering agents is cellulosic materials such as alkylcelluloses (including hydroxyalkylcelluloses), including methylcellulose, ethylcellulose, hydroxyethylcellulose, hydroxypropylcellulose, hydroxypropyl-methylcellulose, and carboxymethylcellulose, the most preferred being cationic salts
30 of carboxymethylcellulose (CMC). Other preferred sequestering agents include hyaluronic acid, sodium alginate, poly(ethylene glycol), polyoxyethylene oxide, carboxyvinyl polymer and poly(vinyl alcohol). The amount of sequestering agent useful herein is 0.5-20 wt%, preferably 1-10 wt% based on total formulation weight, which represents the amount necessary to prevent desorption of the protein from the
35 polymer matrix and to provide appropriate handling of the composition, yet not so much that the progenitor cells are prevented from infiltrating the matrix, thereby

5 providing the protein the opportunity to assist the osteogenic activity of the progenitor cells.

In further compositions, proteins of the invention may be combined with other agents beneficial to the treatment of the bone and/or cartilage defect, wound, or tissue in question. These agents include various growth factors such as epidermal growth factor (EGF), platelet derived growth factor (PDGF), transforming growth factors (TGF- α and TGF- β), and insulin-like growth factor (IGF).
10

The therapeutic compositions are also presently valuable for veterinary applications. Particularly domestic animals and thoroughbred horses, in addition to humans, are desired patients for such treatment with proteins of the present invention.
15

The dosage regimen of a protein-containing pharmaceutical composition to be used in tissue regeneration will be determined by the attending physician considering various factors which modify the action of the proteins, e.g., amount of tissue weight desired to be formed, the site of damage, the condition of the damaged tissue, the size of a wound, type of damaged tissue (e.g., bone), the patient's age, sex, and diet, the severity of any infection, time of administration and other clinical factors. The dosage may vary with the type of matrix used in the reconstitution and with inclusion of other proteins in the pharmaceutical composition. For example, the addition of other known growth factors, such as IGF I (insulin like growth factor I), to the final composition, may also effect the dosage. Progress can be monitored by periodic assessment of tissue/bone growth and/or repair, for example, X-rays, histomorphometric determinations and tetracycline labeling.
20
25

Polynucleotides of the present invention can also be used for gene therapy. Such polynucleotides can be introduced either *in vivo* or *ex vivo* into cells for expression in a mammalian subject. Polynucleotides of the invention may also be administered by other known methods for introduction of nucleic acid into a cell or organism (including, without limitation, in the form of viral vectors or naked DNA).
30

Cells may also be cultured *ex vivo* in the presence of proteins of the present invention in order to proliferate or to produce a desired effect on or activity in such cells. Treated cells can then be introduced *in vivo* for therapeutic purposes.
35

5 Patent and literature references cited herein are incorporated by reference as if fully set forth.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Jacobs, Kenneth
McCoy, John
Kelleher, Kerry
Carlin, McKeough

(ii) TITLE OF INVENTION: DNA SEQUENCES AND SECRETED PROTEINS
ENCODED THEREBY

(iii) NUMBER OF SEQUENCES: 12

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: Genetics Institute, Inc. -- Legal Affairs
(B) STREET: 87 CambridgePark Drive
(C) CITY: Cambridge
(D) STATE: Massachusetts
(E) COUNTRY: USA
(F) ZIP: 02140

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(vii) ATTORNEY/AGENT INFORMATION:

(A) NAME: Brown, Scott A.
(B) REGISTRATION NUMBER: 32,724
(C) REFERENCE/DOCKET NUMBER: GI6000

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: (617) 498-8224
(B) TELEFAX: (617) 876-5851

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 2209 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(ix) FEATURE:

(A) NAME/KEY: CDS
(B) LOCATION: 38..1447

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

| | | | |
|--|-----|-----|-----|
| GAGAAGATAA AACTGGACAC TGGGGAGACA CAACTTC ATG CTG CGT GGG ATC TCC Met Leu Arg Gly Ile Ser | 1 | 5 | 55 |
| CAG CTA CCT GCA GTG GCC ACC ATG TCT TGG GTC CTG CTG CCT GTA CTT Gln Leu Pro Ala Val Ala Thr Met Ser Trp Val Leu Leu Pro Val Leu | 10 | 15 | 20 |
| TGG CTC ATT GTT CAA ACT CAA GCA ATA GCC ATA AAG CAA ACA CCT GAA Trp Leu Ile Val Gln Thr Gln Ala Ile Ala Ile Lys Gln Thr Pro Glu | 25 | 30 | 35 |
| TTA ACG CTC CAT GAA ATA GTT TGT CCT AAA AAA CTT CAC ATT TTA CAC Leu Thr Leu His Glu Ile Val Cys Pro Lys Lys Leu His Ile Leu His | 40 | 45 | 50 |
| AAA AGA GAG ATC AAG AAC AAC CAG ACA GAA AAG CAT GGC AAA GAG GAA Lys Arg Glu Ile Lys Asn Asn Gln Thr Glu Lys His Gly Lys Glu Glu | 55 | 60 | 65 |
| AGG TAT GAA CCT GAA GTT CAA TAT CAG ATG ATC TTA AAT GGA GAA GAA Arg Tyr Glu Pro Glu Val Gln Tyr Gln Met Ile Leu Asn Gly Glu Glu | 75 | 80 | 85 |
| ATC ATT CTC CTA CAA AAA ACC AAG CAC CTC CTG GGG CCA GAC TAC Ile Ile Leu Ser Leu Gln Lys Thr Lys His Leu Leu Gly Pro Asp Tyr | 90 | 95 | 100 |
| ACT GAA ACA TTG TAC TCA CCC AGA GGA GAG GAA ATT ACC ACG AAA CCT Thr Glu Thr Leu Tyr Ser Pro Arg Gly Glu Glu Ile Thr Thr Lys Pro | 105 | 110 | 115 |
| GAG AAC ATG GAA CAC TGT TAC TAT AAA GGA AAC ATC CTA AAT GAA AAG Glu Asn Met Glu His Cys Tyr Tyr Lys Gly Asn Ile Leu Asn Glu Lys | 120 | 125 | 130 |
| AAT TCT GTT GCC AGC ATC AGT ACT TGT GAC GGG TTG AGA GGA TAC TTC Asn Ser Val Ala Ser Ile Ser Thr Cys Asp Gly Leu Arg Gly Tyr Phe | 135 | 140 | 145 |
| ACA CAT CAT CAC CAA AGA TAC CAG ATA AAA CCT CTG AAA AGC ACA GAC Thr His His His Gln Arg Tyr Gln Ile Lys Pro Leu Lys Ser Thr Asp | 155 | 160 | 165 |
| GAG AAA GAA CAT GCC GTC TTT ACA TCT AAC CAG GAG GAA CAA GAC CCA Glu Lys Glu His Ala Val Phe Thr Ser Asn Gln Glu Glu Gln Asp Pro | 170 | 175 | 180 |
| GCT AAC CAC ACA TGT GGT GTG AAG AGC ACT GAC GGG AAA CAA GGC CCA Ala Asn His Thr Cys Gly Val Lys Ser Thr Asp Gly Lys Gln Gly Pro | 185 | 190 | 195 |
| ATT CGA ATC TCT AGA TCA CTC AAA AGC CCA GAG AAA GAA GAC TTT CTT Ile Arg Ile Ser Arg Ser Leu Lys Ser Pro Glu Lys Glu Asp Phe Leu | 200 | 205 | 210 |
| CGG GCA CAG AAA TAC ATT GAT CTC TAT TTG GTG CTG GAT AAT GCC TTT Arg Ala Gln Lys Tyr Ile Asp Leu Tyr Leu Val Leu Asp Asn Ala Phe | 215 | 220 | 225 |
| TAT AAG AAC TAT AAT GAG AAT CTA ACT CTG ATA AGA AGC TTT GTG TTT | | | 775 |

Tyr Lys Asn Tyr Asn Glu Asn Leu Thr Leu Ile Arg Ser Phe Val Phe
235 240 245

| | |
|---|------|
| GAT GTG ATG AAC CTA CTC AAT GTG ATA TAT AAC ACC ATA GAT GTT CAA Asp Val Met Asn Leu Leu Asn Val Ile Tyr Asn Thr Ile Asp Val Gln 250 255 260 | 823 |
| GTG GCC TTG GTA GGT ATG GAA ATC TGG TCT GAT GGG GAT AAG ATA AAG Val Ala Leu Val Gly Met Glu Ile Trp Ser Asp Gly Asp Lys Ile Lys 265 270 275 | 871 |
| GTG GTG CCC AGC GCA AGC ACC ACG TTT GAC AAC TTC CTG AGA TGG CAC Val Val Pro Ser Ala Ser Thr Thr Phe Asp Asn Phe Leu Arg Trp His 280 285 290 | 919 |
| AGT TCT AAC CTG GGG AAA AAG ATC CAC GAC CAT GCT CAG CTT CTC AGC Ser Ser Asn Leu Gly Lys Lys Ile His Asp His Ala Gln Leu Leu Ser 295 300 305 310 | 967 |
| GGG ATT AGC TTC AAC AAT CGA CGT GTG GGA CTG GCA GCT TCA AAT TCC Gly Ile Ser Phe Asn Asn Arg Arg Val Gly Leu Ala Ala Ser Asn Ser 315 320 325 | 1015 |
| TTG TGT TCC CCA TCT TCG GTT GCT ATT GAG GCT AAA AAA AAG AAT Leu Cys Ser Pro Ser Ser Val Ala Val Ile Glu Ala Lys Lys Lys Asn 330 335 340 | 1063 |
| AAT GTG GCT CTT GTA GGA GTG ATG TCA CAT GAG CTG GGC CAT GTC CTT Asn Val Ala Leu Val Gly Val Met Ser His Glu Leu Gly His Val Leu 345 350 355 | 1111 |
| GGT ATG CCT GAT GTT CCA TTC AAC ACC AAG TGT CCC TCT GGC AGT TGT Gly Met Pro Asp Val Pro Phe Asn Thr Lys Cys Pro Ser Gly Ser Cys 360 365 370 | 1159 |
| GTG ATG AAT CAG TAT CTG AGT TCA AAA TTC CCA AAG GAT TTC AGT ACA Val Met Asn Gln Tyr Leu Ser Ser Lys Phe Pro Lys Asp Phe Ser Thr 375 380 385 390 | 1207 |
| TCT TGC CGT GCA CAT TTT GAA AGA TAC CTT TTA TCT CAG AAA CCA AAG Ser Cys Arg Ala His Phe Glu Arg Tyr Leu Leu Ser Gln Lys Pro Lys 395 400 405 | 1255 |
| TGC CTG CTG CAA GCA CCT ATT CCT ACA AAT ATA ATG ACA ACA CCA GTG Cys Leu Leu Gln Ala Pro Ile Pro Thr Asn Ile Met Thr Thr Pro Val 410 415 420 | 1303 |
| TGT GGG AAC CAC CTT CTA GAA GTG GGA GAA GAC TGT GAT TGT GGC TCT Cys Gly Asn His Leu Leu Glu Val Gly Glu Asp Cys Asp Cys Gly Ser 425 430 435 | 1351 |
| CCT AAG GAG TGT ACC AAT CTC TGC TGT GAA GCC CTA ACG TGT AAA CTG Pro Lys Glu Cys Thr Asn Leu Cys Cys Glu Ala Leu Thr Cys Lys Leu 440 445 450 | 1399 |
| AAG CCT GGA ACT GAT TGC GGA GGA GAT GCT CCA AAC CAT ACC ACA GAG Lys Pro Gly Thr Asp Cys Gly Gly Asp Ala Pro Asn His Thr Thr Glu 455 460 465 470 | 1447 |
| TGAATCCAAA AGTCTGCTTC ACTGAGATGC TACCTTGCCA GGACAAGAAC CAAGAACTCT | 1507 |
| AACTGTCCCA GGAATCTTGT GAATTTCAC CCATAATGGT CTTTCACTTG TCATTCTACT | 1567 |
| TTCTATATTG TTATCAGTCC AGGAAACAGG TAAACAGATG TAATTAGAGA CATTGGCTCT | 1627 |
| TTGTTTAGGC CTAATCTTTC TTTTTACTTT TTTTTTTCTT TTTTCTTTT TTTTAAAGAT | 1687 |

| | |
|---|------|
| CATGAATTTG TGACTTAGTT CTGCCCTTG GAGAACAAAA GAAAGCAGTC TTCCATCAAA | 1747 |
| TCACCTTAAA ATGCACGGCT AACTATTCA GAGTTAACAC TCCAGAATTG TTAAATTACA | 1807 |
| AGTACTATGC TTTAATGCTT CTTCATCTT ACTAGTATGG CCTATAAAA AAATAATACC | 1867 |
| ACTTGATGGG TGAAGGCTTT GGCAATAGAA AGAAGAATAG AATTCAAGGT TTATGTTATT | 1927 |
| CCTCTGTGTT CACTTCGCCT TGCTCTGAA AGTGCAGTAT TTTTCTACAT CATGTCGAGA | 1987 |
| ATGATTCAAT GTAAATATTTC TTCATTTAT CATGTATATC CTATACACAC ATCTCCTTCA | 2047 |
| TCATCATATA TGAAGTTTAT TTTGAGAAAGT CTACATTGCT TACATTTAA TTGAGCCAGC | 2107 |
| AAAGAAGGCT TAATGATTAA TTGAACCATA ATGTCATAAA AACACACAAT TTTGAGGCAA | 2167 |
| AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AA | 2209 |

(2) INFORMATION FOR SEQ ID NO:2:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 470 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

| | | | |
|---|-----|-----|-----|
| Met Leu Arg Gly Ile Ser Gln Leu Pro Ala Val Ala Thr Met Ser Trp | | | |
| 1 | 5 | 10 | 15 |
| Val Leu Leu Pro Val Leu Trp Leu Ile Val Gln Thr Gln Ala Ile Ala | | | |
| 20 | 25 | 30 | |
| Ile Lys Gln Thr Pro Glu Leu Thr Leu His Glu Ile Val Cys Pro Lys | | | |
| 35 | 40 | 45 | |
| Lys Leu His Ile Leu His Lys Arg Glu Ile Lys Asn Asn Gln Thr Glu | | | |
| 50 | 55 | 60 | |
| Lys His Gly Lys Glu Glu Arg Tyr Glu Pro Glu Val Gln Tyr Gln Met | | | |
| 65 | 70 | 75 | 80 |
| Ile Leu Asn Gly Glu Ile Ile Leu Ser Leu Gln Lys Thr Lys His | | | |
| 85 | 90 | 95 | |
| Leu Leu Gly Pro Asp Tyr Thr Glu Thr Leu Tyr Ser Pro Arg Gly Glu | | | |
| 100 | 105 | 110 | |
| Glu Ile Thr Thr Lys Pro Glu Asn Met Glu His Cys Tyr Tyr Lys Gly | | | |
| 115 | 120 | 125 | |
| Asn Ile Leu Asn Glu Lys Asn Ser Val Ala Ser Ile Ser Thr Cys Asp | | | |
| 130 | 135 | 140 | |
| Gly Leu Arg Gly Tyr Phe Thr His His His Gln Arg Tyr Gln Ile Lys | | | |
| 145 | 150 | 155 | 160 |
| Pro Leu Lys Ser Thr Asp Glu Lys Glu His Ala Val Phe Thr Ser Asn | | | |
| 165 | 170 | 175 | |

Gln Glu Glu Gln Asp Pro Ala Asn His Thr Cys Gly Val Lys Ser Thr
 180 185 190
 Asp Gly Lys Gln Gly Pro Ile Arg Ile Ser Arg Ser Leu Lys Ser Pro
 195 200 205
 Glu Lys Glu Asp Phe Leu Arg Ala Gln Lys Tyr Ile Asp Leu Tyr Leu
 210 215 220
 Val Leu Asp Asn Ala Phe Tyr Lys Asn Tyr Asn Glu Asn Leu Thr Leu
 225 230 235 240
 Ile Arg Ser Phe Val Phe Asp Val Met Asn Leu Leu Asn Val Ile Tyr
 245 250 255
 Asn Thr Ile Asp Val Gln Val Ala Leu Val Gly Met Glu Ile Trp Ser
 260 265 270
 Asp Gly Asp Lys Ile Lys Val Val Pro Ser Ala Ser Thr Thr Phe Asp
 275 280 285
 Asn Phe Leu Arg Trp His Ser Ser Asn Leu Gly Lys Lys Ile His Asp
 290 295 300
 His Ala Gln Leu Leu Ser Gly Ile Ser Phe Asn Asn Arg Arg Val Gly
 305 310 315 320
 Leu Ala Ala Ser Asn Ser Leu Cys Ser Pro Ser Ser Val Ala Val Ile
 325 330 335
 Glu Ala Lys Lys Lys Asn Asn Val Ala Leu Val Gly Val Met Ser His
 340 345 350
 Glu Leu Gly His Val Leu Gly Met Pro Asp Val Pro Phe Asn Thr Lys
 355 360 365
 Cys Pro Ser Gly Ser Cys Val Met Asn Gln Tyr Leu Ser Ser Lys Phe
 370 375 380
 Pro Lys Asp Phe Ser Thr Ser Cys Arg Ala His Phe Glu Arg Tyr Leu
 385 390 395 400
 Leu Ser Gln Lys Pro Lys Cys Leu Leu Gln Ala Pro Ile Pro Thr Asn
 405 410 415
 Ile Met Thr Thr Pro Val Cys Gly Asn His Leu Leu Glu Val Gly Glu
 420 425 430
 Asp Cys Asp Cys Gly Ser Pro Lys Glu Cys Thr Asn Leu Cys Cys Glu
 435 440 445
 Ala Leu Thr Cys Lys Leu Lys Pro Gly Thr Asp Cys Gly Gly Asp Ala
 450 455 460
 Pro Asn His Thr Thr Glu
 465 470

(2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 2582 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(ix) FEATURE:

(A) NAME/KEY: CDS
(B) LOCATION: 52..2034

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

ATTTCTCAGC TCCAAGCATT AGGTAAACCC ACCAAGCAAT CCTAGCCTGT G ATG GCG
 Met Ala
 1

TTT GAC GTC AGC TGC TTC TTT TGG GTG GTG CTG TTT TCT GCC GGC TGT
 Phe Asp Val Ser Cys Phe Phe Trp Val Val Leu Phe Ser Ala Gly Cys
 5 10 15

AAA GTC ATC ACC TCC TGG GAT CAG ATG TGC ATT GAG AAA GAA GCC AAC
 Lys Val Ile Thr Ser Trp Asp Gln Met Cys Ile Glu Lys Glu Ala Asn
 20 25 30

AAA ACA TAT AAC TGT GAA AAT TTA GGT CTC AGT GAA ATC CCT GAC ACT
 Lys Thr Tyr Asn Cys Glu Asn Leu Gly Leu Ser Glu Ile Pro Asp Thr
 35 40 45 50

CTA CCA AAC ACA ACA GAA TTT TTG GAA TTC AGC TTT AAT TTT TTG CCT
 Leu Pro Asn Thr Thr Glu Phe Leu Glu Phe Ser Phe Asn Phe Leu Pro
 55 60 65

ACA ATT CAC AAT AGA ACC TTC AGC AGA CTC ATG AAT CTT ACC TTT TTG
 Thr Ile His Asn Arg Thr Phe Ser Arg Leu Met Asn Leu Thr Phe Leu
 70 75 80

GAT TTA ACT AGG TGC CAG ATT AAC TGG ATA CAT GAA GAC ACT TTT CAA
 Asp Leu Thr Arg Cys Gln Ile Asn Trp Ile His Glu Asp Thr Phe Gln
 85 90 95

AGC CAT CAT CAA TTA AGC ACA CTT GTG TTA ACT GGA AAT CCC CTG ATA
 Ser His His Gln Leu Ser Thr Leu Val Leu Thr Gly Asn Pro Leu Ile
 100 105 110

TTC ATG GCA GAA ACA TCG CTT AAT GGG CCC AAG TCA CTG AAG CAT CTT
 Phe Met Ala Glu Thr Ser Leu Asn Gly Pro Lys Ser Leu Lys His Leu
 115 120 125 130

TTC TTA ATC CAA ACG GGA ATA TCC AAT CTC GAG TTT ATT CCA GTG CAC
 Phe Leu Ile Gln Thr Gly Ile Ser Asn Leu Glu Phe Ile Pro Val His
 135 140 145

AAT CTG GAA AAC TTG GAA AGC TTG TAT CTT GGA AGC AAC CAT ATT TCC
 Asn Leu Glu Asn Leu Glu Ser Leu Tyr Leu Gly Ser Asn His Ile Ser
 150 155 160

TCC ATT AAG TTC CCC AAA GAC TTC CCA GCA CGG AAT CTG AAA GTA CTG
 Ser Ile Lys Phe Pro Lys Asp Phe Pro Ala Arg Asn Leu Lys Val Leu
 165 170 175

GAT TTT CAG AAT AAT GCT ATA CAC TAC ATC TCT AGA GAA GAC ATG AGG

| | | |
|---|--|------|
| Asp Phe Gln Asn Asn Ala Ile His Tyr Ile Ser Arg Glu Asp Met Arg 180 185 190 | | |
| TCT CTG GAG CAG GCC ATC AAC CTA AGC CTG AAC TTC AAT GGC AAT AAT Ser Leu Glu Gln Ala Ile Asn Leu Ser Leu Asn Phe Asn Gly Asn Asn 195 200 205 210 | | 681 |
| GTT AAA GGT ATT GAG CTT GGG GCT TTT GAT TCA ACG GTC TTC CAA AGT Val Lys Gly Ile Glu Leu Gly Ala Phe Asp Ser Thr Val Phe Gln Ser 215 220 225 | | 729 |
| TTG AAC TTT GGA GGA ACT CCA AAT TTG TCT ATA TTC AAT GGT CTG Leu Asn Phe Gly Gly Thr Pro Asn Leu Ser Val Ile Phe Asn Gly Leu 230 235 240 | | 777 |
| CAG AAC TCT ACT ACT CAG TCT CTC TGG CTG GGA ACA TTT GAG GAC ATT Gln Asn Ser Thr Thr Gln Ser Leu Trp Leu Gly Thr Phe Glu Asp Ile 245 250 255 | | 825 |
| GAT GAC GAA GAT ATT AGT TCA GCC ATG CTC AAG GGA CTC TGT GAA ATG Asp Asp Glu Asp Ile Ser Ser Ala Met Leu Lys Gly Leu Cys Glu Met 260 265 270 | | 873 |
| TCT GTT GAG AGC CTC AAC CTG CAG GAA CAC CGC TTC TCT GAC ATC TCA Ser Val Glu Ser Leu Asn Leu Gln Glu His Arg Phe Ser Asp Ile Ser 275 280 285 290 | | 921 |
| TCC ACC ACA TTT CAG TGC TTC ACC CAA CTC CAA GAA TTG GAT CTG ACA Ser Thr Thr Phe Gln Cys Phe Thr Gln Leu Gln Glu Leu Asp Leu Thr 295 300 305 | | 969 |
| GCA ACT CAC TTG AAA GGG TTA CCC TCT GGG ATG AAG GGT CTG AAC TTG Ala Thr His Leu Lys Gly Leu Pro Ser Gly Met Lys Gly Leu Asn Leu 310 315 320 | | 1017 |
| CTC AAG AAA TTA GTT CTC AGT GTA AAT CAT TTC GAT CAA TTG TGT CAA Leu Lys Lys Leu Val Leu Ser Val Asn His Phe Asp Gln Leu Cys Gln 325 330 335 | | 1065 |
| ATC AGT GCT GCC AAT TTC CCC TCC CTT ACA CAC CTC TAC ATC AGA GGC Ile Ser Ala Ala Asn Phe Pro Ser Leu Thr His Leu Tyr Ile Arg Gly 340 345 350 | | 1113 |
| AAC GTG AAG AAA CTT CAC CTT GGT GTT GGC TGC TTG GAG AAA CTA GGA Asn Val Lys Lys Leu His Leu Gly Val Gly Cys Leu Glu Lys Leu Gly 355 360 365 370 | | 1161 |
| AAC CTT CAG ACA CTT GAT TTA AGC CAT AAT GAC ATA GAG GCT TCT GAC Asn Leu Gln Thr Leu Asp Leu Ser His Asn Asp Ile Glu Ala Ser Asp 375 380 385 | | 1209 |
| TGC TGC AGT CTG CAA CTC AAA AAC CTG TCC CAC TTG CAA ACC TTA AAC Cys Cys Ser Leu Gln Leu Lys Asn Leu Ser His Leu Gln Thr Leu Asn 390 395 400 | | 1257 |
| CTG AGC CAC AAT GAG CCT CTT GGT CTC CAG AGT CAG GCA TTC AAA GAA Leu Ser His Asn Glu Pro Leu Gly Leu Gln Ser Gln Ala Phe Lys Glu 405 410 415 | | 1305 |
| TGT CCT CAG CTA GAA CTC CTC GAT TTG GCA TTT ACC CGC TTA CAC ATT Cys Pro Gln Leu Glu Leu Leu Asp Leu Ala Phe Thr Arg Leu His Ile 420 425 430 | | 1353 |

| | |
|---|------|
| AAT GCT CCA CAA AGT CCC TTC CAA AAC CTC CAT TTC CTT CAG GTT CTG Asn Ala Pro Gln Ser Pro Phe Gln Asn Leu His Phe Leu Gln Val Leu 435 440 445 450 | 1401 |
| AAT CTC ACT TAC TGC TTC CTT GAT ACC AGC AAT CAG CAT CTT CTA GCA Asn Leu Thr Tyr Cys Phe Leu Asp Thr Ser Asn Gln His Leu Leu Ala 455 460 465 | 1449 |
| GGC CTA CCA GTT CTC CGG CAT CTC AAC TTA AAA GGG AAT CAC TTT CAA Gly Leu Pro Val Leu Arg His Leu Asn Leu Lys Gly Asn His Phe Gln 470 475 480 | 1497 |
| GAT GGG ACT ATC ACG AAG ACC AAC CTA CTT CAG ACC GTG GGC AGC TTG Asp Gly Thr Ile Thr Lys Thr Asn Leu Leu Gln Thr Val Gly Ser Leu 485 490 495 | 1545 |
| GAG GTT CTG ATT TTG TCC TCT TGT GGT CTC CTC TCT ATA GAC CAG CAA Glu Val Leu Ile Leu Ser Ser Cys Gly Leu Leu Ser Ile Asp Gln Gln 500 505 510 | 1593 |
| GCA TTC CAC AGC TTG GGA AAA ATG AGC CAT GTA GAC TTA AGC CAC AAC Ala Phe His Ser Leu Gly Lys Met Ser His Val Asp Leu Ser His Asn 515 520 525 530 | 1641 |
| AGC CTG ACA TGC GAC AGC ATT GAT TCT CTT AGC CAT CTT AAG GGA ATC Ser Leu Thr Cys Asp Ser Ile Asp Ser Leu Ser His Leu Lys Gly Ile 535 540 545 | 1689 |
| TAC CTC AAT CTG GCT GCC AAC AGC ATT AAC ATC ATC TCA CCC CGT CTC Tyr Leu Asn Leu Ala Ala Asn Ser Ile Asn Ile Ile Ser Pro Arg Leu 550 555 560 | 1737 |
| CTC CCT ATC TTG TCC CAG CAG AGC ACC ATT AAT TTA AGT CAT AAC CCC Leu Pro Ile Leu Ser Gln Gln Ser Thr Ile Asn Leu Ser His Asn Pro 565 570 575 | 1785 |
| CTG GAC TGC ACT TGC TCG AAT ATT CAT TTC TTA ACA TGG TAC AAA GAA Leu Asp Cys Thr Cys Ser Asn Ile His Phe Leu Thr Trp Tyr Lys Glu 580 585 590 | 1833 |
| AAC CTG CAC AAA CTT GAA GGC TCG GAG GAG ACC ACG TGT GCA AAC CCG Asn Leu His Leu Glu Gly Ser Glu Glu Thr Thr Cys Ala Asn Pro 595 600 605 610 | 1881 |
| CCA TCT CTA AGG GGA GTT AAG CTA TCT GAT GTC AAG CTT TCC TGT GGG Pro Ser Leu Arg Gly Val Lys Leu Ser Asp Val Lys Leu Ser Cys Gly 615 620 625 | 1929 |
| ATT ACA GCC ATA GGC ATT TTC TTT CTC ATA GTA TTT CTA TTA TTG TTG Ile Thr Ala Ile Gly Ile Phe Phe Leu Ile Val Phe Leu Leu Leu 630 635 640 | 1977 |
| GCT ATT CTG CTA TTT TTT GCA GTT AAA TAC CTT CTC AGG TGG AAA TAC Ala Ile Leu Leu Phe Phe Ala Val Lys Tyr Leu Leu Arg Trp Lys Tyr 645 650 655 | 2025 |
| CAA CAC ATT TAGTGCTGAA GGTTCCAGA GAAAGCAAAT AAGTGTGCTT Gln His Ile 660 | 2074 |
| AGCAAAATTG CTCTAAGTGA AAGAACTGTC ATCTGCTGGT GACCAGACCA GACTTTTCAG | 2134 |
| ATTGCTTCCT GGAACGGGC AGGGACTCAC TGTGCTTTTC TGAGCTTCTT ACTCCTGTGA | 2194 |

| | | | | | |
|-----------------------|------------|------------|------------|------------|------|
| GTCCCAGAGC TAAAGAACCT | TCTAGGCAAG | TACACCGAAT | GACTCAGTCC | AGAGGGTCAG | 2254 |
| ATGCTGCTGT | GAGAGGCACA | GAGCCCTTTC | CGCATGTGGA | AGAGTGGGAG | 2314 |
| GAGGGACTGG | GCAGGGACTG | CCGGCCCCGG | AGTCTCCAC | AGGGAGGCCA | 2374 |
| ACTCACCGAC | ATCCCTCCA | GCACCACACA | CCCCGCCCC | GAAAGGAGAT | 2434 |
| CACAATTGT | CAGAGCTGAA | GCCAGCCCAC | TACCCACCCC | CACTACAGCA | 2494 |
| GTCTGGGTTC | TCAGTAATGT | AGCCATTGA | GAAACTTACT | TGGGGACAAA | 2554 |
| TTATTTAAA | TGAAAAAAA | AAAAAAA | | | 2582 |

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 661 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

| | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Met | Ala | Phe | Asp | Val | Ser | Cys | Phe | Phe | Trp | Val | Val | Leu | Phe | Ser | Ala |
| 1 | | | | 5 | | | | | | 10 | | | | | 15 |
| Gly | Cys | Lys | Val | Ile | Thr | Ser | Trp | Asp | Gln | Met | Cys | Ile | Glu | Lys | Glu |
| | | | | 20 | | | | | 25 | | | | | 30 | |
| Ala | Asn | Lys | Thr | Tyr | Asn | Cys | Glu | Asn | Leu | Gly | Leu | Ser | Glu | Ile | Pro |
| | | | | 35 | | | | 40 | | | | | | 45 | |
| Asp | Thr | Leu | Pro | Asn | Thr | Thr | Glu | Phe | Leu | Glu | Ser | Phe | Asn | Phe | |
| | | | | 50 | | | 55 | | | | | | | 60 | |
| Leu | Pro | Thr | Ile | His | Asn | Arg | Thr | Phe | Ser | Arg | Leu | Met | Asn | Leu | Thr |
| | | | | 65 | | | 70 | | | 75 | | | | 80 | |
| Phe | Leu | Asp | Leu | Thr | Arg | Cys | Gln | Ile | Asn | Trp | Ile | His | Glu | Asp | Thr |
| | | | | 85 | | | | 90 | | | | | | 95 | |
| Phe | Gln | Ser | His | His | Gln | Leu | Ser | Thr | Leu | Val | Leu | Thr | Gly | Asn | Pro |
| | | | | 100 | | | | 105 | | | | | | 110 | |
| Leu | Ile | Phe | Met | Ala | Glu | Thr | Ser | Leu | Asn | Gly | Pro | Lys | Ser | Leu | Lys |
| | | | | 115 | | | | 120 | | | | | | 125 | |
| His | Leu | Phe | Leu | Ile | Gln | Thr | Gly | Ile | Ser | Asn | Leu | Glu | Phe | Ile | Pro |
| | | | | 130 | | | | 135 | | | | | | 140 | |
| Val | His | Asn | Leu | Glu | Asn | Leu | Glu | Ser | Leu | Tyr | Leu | Gly | Ser | Asn | His |
| | | | | 145 | | | | 150 | | | 155 | | | 160 | |
| Ile | Ser | Ser | Ile | Lys | Phe | Pro | Lys | Asp | Phe | Pro | Ala | Arg | Asn | Leu | Lys |
| | | | | 165 | | | | 170 | | | | | | 175 | |
| Val | Leu | Asp | Phe | Gln | Asn | Asn | Ala | Ile | His | Tyr | Ile | Ser | Arg | Glu | Asp |
| | | | | 180 | | | | 185 | | | | | | 190 | |
| Met | Arg | Ser | Leu | Glu | Gln | Ala | Ile | Asn | Leu | Ser | Leu | Asn | Phe | Asn | Gly |

| 195 | 200 | 205 |
|---|-----|-----|
| Asn Asn Val Lys Gly Ile Glu Leu Gly Ala Phe Asp Ser Thr Val Phe | | |
| 210 | 215 | 220 |
| Gln Ser Leu Asn Phe Gly Gly Thr Pro Asn Leu Ser Val Ile Phe Asn | | |
| 225 | 230 | 235 |
| 240 | | |
| Gly Leu Gln Asn Ser Thr Thr Gln Ser Leu Trp Leu Gly Thr Phe Glu | | |
| 245 | 250 | 255 |
| Asp Ile Asp Asp Glu Asp Ile Ser Ser Ala Met Leu Lys Gly Leu Cys | | |
| 260 | 265 | 270 |
| Glu Met Ser Val Glu Ser Leu Asn Leu Gln Glu His Arg Phe Ser Asp | | |
| 275 | 280 | 285 |
| Ile Ser Ser Thr Thr Phe Gln Cys Phe Thr Gln Leu Gln Glu Leu Asp | | |
| 290 | 295 | 300 |
| Leu Thr Ala Thr His Leu Lys Gly Leu Pro Ser Gly Met Lys Gly Leu | | |
| 305 | 310 | 315 |
| 320 | | |
| Asn Leu Leu Lys Lys Leu Val Leu Ser Val Asn His Phe Asp Gln Leu | | |
| 325 | 330 | 335 |
| Cys Gln Ile Ser Ala Ala Asn Phe Pro Ser Leu Thr His Leu Tyr Ile | | |
| 340 | 345 | 350 |
| Arg Gly Asn Val Lys Lys Leu His Leu Gly Val Gly Cys Leu Glu Lys | | |
| 355 | 360 | 365 |
| Leu Gly Asn Leu Gln Thr Leu Asp Leu Ser His Asn Asp Ile Glu Ala | | |
| 370 | 375 | 380 |
| Ser Asp Cys Cys Ser Leu Gln Leu Lys Asn Leu Ser His Leu Gln Thr | | |
| 385 | 390 | 395 |
| 400 | | |
| Leu Asn Leu Ser His Asn Glu Pro Leu Gly Leu Gln Ser Gln Ala Phe | | |
| 405 | 410 | 415 |
| Lys Glu Cys Pro Gln Leu Glu Leu Leu Asp Leu Ala Phe Thr Arg Leu | | |
| 420 | 425 | 430 |
| His Ile Asn Ala Pro Gln Ser Pro Phe Gln Asn Leu His Phe Leu Gln | | |
| 435 | 440 | 445 |
| Val Leu Asn Leu Thr Tyr Cys Phe Leu Asp Thr Ser Asn Gln His Leu | | |
| 450 | 455 | 460 |
| Leu Ala Gly Leu Pro Val Leu Arg His Leu Asn Leu Lys Gly Asn His | | |
| 465 | 470 | 475 |
| 480 | | |
| Phe Gln Asp Gly Thr Ile Thr Lys Thr Asn Leu Leu Gln Thr Val Gly | | |
| 485 | 490 | 495 |
| Ser Leu Glu Val Leu Ile Leu Ser Ser Cys Gly Leu Leu Ser Ile Asp | | |
| 500 | 505 | 510 |
| Gln Gln Ala Phe His Ser Leu Gly Lys Met Ser His Val Asp Leu Ser | | |
| 515 | 520 | 525 |
| His Asn Ser Leu Thr Cys Asp Ser Ile Asp Ser Leu Ser His Leu Lys | | |

| | | |
|---|-----|-----|
| 530 | 535 | 540 |
| Gly Ile Tyr Leu Asn Leu Ala Ala Asn Ser Ile Asn Ile Ile Ser Pro | | |
| 545 | 550 | 555 |
| 560 | | |
| Arg Leu Leu Pro Ile Leu Ser Gln Gln Ser Thr Ile Asn Leu Ser His | | |
| 565 | 570 | 575 |
| Asn Pro Leu Asp Cys Thr Cys Ser Asn Ile His Phe Leu Thr Trp Tyr | | |
| 580 | 585 | 590 |
| Lys Glu Asn Leu His Lys Leu Glu Gly Ser Glu Glu Thr Thr Cys Ala | | |
| 595 | 600 | 605 |
| Asn Pro Pro Ser Leu Arg Gly Val Lys Leu Ser Asp Val Lys Leu Ser | | |
| 610 | 615 | 620 |
| Cys Gly Ile Thr Ala Ile Gly Ile Phe Phe Leu Ile Val Phe Leu Leu | | |
| 625 | 630 | 635 |
| 640 | | |
| Leu Leu Ala Ile Leu Leu Phe Phe Ala Val Lys Tyr Leu Leu Arg Trp | | |
| 645 | 650 | 655 |
| Lys Tyr Gln His Ile | | |
| 660 | | |

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 588 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 76..474

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

| | |
|--|-----|
| CGGCCAAAGA GGCCTAAACT TGC GGCTGTC CATCTCACCT ACAGCTCTGG TCTCATCCTC | 60 |
| AACTCAACCA CAATC ATG GCT CAG ATG ACT CTG AGC CTC CTT AGC CTG | 111 |
| Met Ala Gln Met Met Thr Leu Ser Leu Ser Leu | |
| 1 5 10 | |
| GTC CTG GCT CTC TGC ATC CCC TGG ACC CAA GGC AGT GAT GGA GGG GGT | 159 |
| Val Leu Ala Leu Cys Ile Pro Trp Thr Gln Gly Ser Asp Gly Gly Gly | |
| 15 20 25 | |
| CAG GAC TGC TGC CTT AAG TAC AGC CAG AAG AAA ATT CCC TAC AGT ATT | 207 |
| Gln Asp Cys Cys Leu Lys Tyr Ser Gln Lys Lys Ile Pro Tyr Ser Ile | |
| 30 35 40 | |
| GTC CGA GGC TAT AGG AAG CAA GAA CCA AGT TTA GGC TGT CCC ATC CCG | 255 |
| Val Arg Gly Tyr Arg Lys Gln Glu Pro Ser Leu Gly Cys Pro Ile Pro | |
| 45 50 55 60 | |

| | |
|--|-----|
| GCA ATC CTG TTC TCA CCC CGG AAG CAC TCT AAG CCT GAG CTA TGT GCA Ala Ile Leu Phe Ser Pro Arg Lys His Ser Lys Pro Glu Leu Cys Ala | 303 |
| 65 70 75 | |
| AAC CCT GAG GAA GGC TGG GTG CAG AAC CTG ATG CGC CGC CTG GAC CAG Asn Pro Glu Glu Gly Trp Val Gln Asn Leu Met Arg Arg Leu Asp Gln | 351 |
| 80 85 90 | |
| CCT CCA GCC CCA GGG AAA CAA AGC CCC GGC TGC AGG AAG AAC CGG GGA Pro Pro Ala Pro Gly Lys Gln Ser Pro Gly Cys Arg Lys Asn Arg Gly | 399 |
| 95 100 105 | |
| ACC TCT AAG TCT GGA AAG AAA GGA AAG GGC TCC AAG GGC TGC AAG AGA Thr Ser Lys Ser Gly Lys Lys Gly Lys Ser Lys Gly Cys Lys Arg | 447 |
| 110 115 120 | |
| ACT GAA CAG ACA CAG CCC TCA AGA GGA TAGCCCCAGTA GCCCGCCTGG Thr Glu Gln Thr Gln Pro Ser Arg Gly | 494 |
| 125 130 | |
| AGCCCCAGGAG ATCCCCCACG AACTTCAAGC TGGGTGGTTC ACGGTCCAAC TCACAGGCCA | 554 |
| AGAGGGAGCT AGAAAAACAGA CTCAGGAGGCC GCTA | 588 |

(2) INFORMATION FOR SEQ ID NO:6:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 133 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear
 - ii) MOLECULE TYPE: protein
 - xii) SEQUENCE DESCRIPTION: SEQ ID NO:6:

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 966 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: double
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(ix) FEATURE:

(A) NAME/KEY: CDS
 (B) LOCATION: 67..348

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

| | |
|--|-----|
| CTTCCAAGAA GAGCAGCAAA GCTGAAGTAG CAGAACAGC ACCAGCAGCA ACAGCAAAAA | 60 |
| ACAAAC ATG AGT GTG AAG GGC ATG GCT ATA GCC TTG GCT GTG ATA TTG Met Ser Val Lys Gly Met Ala Ile Ala Leu Ala Val Ile Leu | 108 |
| 1 5 10 | |
| TGT GCT ACA GTT GTT CAA GGC TTC CCC ATG TTC AAA AGA GGA CGC TGT Cys Ala Thr Val Val Gln Gly Phe Pro Met Phe Lys Arg Gly Arg Cys | 156 |
| 15 20 25 30 | |
| CTT TGC ATA GGC CCT GGG GTA AAA GCA GTG AAA GTG GCA GAT ATT GAG Leu Cys Ile Gly Pro Gly Val Lys Ala Val Lys Val Ala Asp Ile Glu | 204 |
| 35 40 45 | |
| AAA GCC TCC ATA ATG TAC CCA AGT AAC AAC TGT GAC AAA ATA GAA GTG Lys Ala Ser Ile Met Tyr Pro Ser Asn Asn Cys Asp Lys Ile Glu Val | 252 |
| 50 55 60 | |
| ATT ATT ACC CTG AAA GAA AAT AAA GGA CAA CGA TGC CTA AAT CCC AAA Ile Ile Thr Leu Lys Glu Asn Lys Gly Gln Arg Cys Leu Asn Pro Lys | 300 |
| 65 70 75 | |
| TCG AAG CAA GCA AGG CTT ATA ATC AAA AAA GTT GAA AGA AAG AAT TTT Ser Lys Gln Ala Arg Leu Ile Ile Lys Lys Val Glu Arg Lys Asn Phe | 348 |
| 80 85 90 | |
| TAAAAATATC AAAACATATG AAGTCCTGGA AAAGGGCATC TGAAAAACCT AGAACAAAGTT | 408 |
| TAACGTGAC TACTGAAATG ACAAGAATTG TACAGTAGGA AACTGAGACT TTTCTATGGT | 468 |
| TTTGTGACTT TCAACTTTTG TACAGTTATG TGAAGGATGA AAGGTGGGTG AAAGGACCAA | 528 |
| AAACAGAAAT ACAGTCTTCC TGAATGAATG ACAATCAGAA TTCCACTGCC CAAAGGAGTC | 588 |
| CAACAATTAA ATGGATTCT AGGAAAAGCT ACCTTAAGAA AGGCTGGTTA CCATCGGAGT | 648 |
| TTACAAAGTG CTTTCACGTT CTTACTTGT GTATTATAACA TTCATGCATT TCTAGGCTAG | 708 |
| AGAACCTTCT AGATTTGATG CTTACAACTA TTCTGTTGTG ACTATGAGAA CATTCTGTC | 768 |
| TCTAGAAGTT ATCTGTCTGT ATTGATCTTT ATGCTATATT ACTATCTGTG GTTACAGTGG | 828 |
| AGACATTGAC ATTATTACTG GAGTCAAGCC CTTATAAGTC AAAAGCACCT ATGTGTCGTA | 888 |

| | |
|--|-----|
| AAGCATTCCCT CAAACATTTA AAAAAAAA AAAAAAAA AAAAAAAA AAAAAAAA | 948 |
| AAAAAAAAA AAAAAAAA | 966 |

(2) INFORMATION FOR SEQ ID NO:8:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 94 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

| | | | |
|---|---|----|----|
| Met Ser Val Lys Gly Met Ala Ile Ala Leu Ala Val Ile Leu Cys Ala | | | |
| 1 | 5 | 10 | 15 |

| | | |
|---|----|----|
| Thr Val Val Gln Gly Phe Pro Met Phe Lys Arg Gly Arg Cys Leu Cys | | |
| 20 | 25 | 30 |

| | | |
|---|----|----|
| Ile Gly Pro Gly Val Lys Ala Val Lys Val Ala Asp Ile Glu Lys Ala | | |
| 35 | 40 | 45 |

| | | |
|---|----|----|
| Ser Ile Met Tyr Pro Ser Asn Asn Cys Asp Lys Ile Glu Val Ile Ile | | |
| 50 | 55 | 60 |

| | | | |
|---|----|----|----|
| Thr Leu Lys Glu Asn Lys Gly Gln Arg Cys Leu Asn Pro Lys Ser Lys | | | |
| 65 | 70 | 75 | 80 |

| | |
|---|----|
| Gln Ala Arg Leu Ile Ile Lys Lys Val Glu Arg Lys Asn Phe | |
| 85 | 90 |

(2) INFORMATION FOR SEQ ID NO:9:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 1354 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 75..356

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

| | |
|---|----|
| TTCTACTCCT TCCAAGAAGA GCAGCAAAGC TGAAGTAGCA GCAACAGCAC CAGCAGCAAC | 60 |
|---|----|

| | | |
|---|-----|-----|
| AGCAAAAAAC AAAC ATG AGT GTG AAG GGC ATG GCT ATA GCC TTG GCT GTG | 110 | |
| Met Ser Val Lys Gly Met Ala Ile Ala Leu Ala Val | | |
| 1 | 5 | 10. |

| | | |
|---|-----|----|
| ATA TTG TGT GCT ACA GTT GTT CAA GGC TTC CCC ATG TTC AAA AGA GGA | 158 | |
| Ile Leu Cys Ala Thr Val Val Gln Gly Phe Pro Met Phe Lys Arg Gly | | |
| 15 | 20 | 25 |

| | |
|---|---|
| CGC TGT CTT TGC ATA GGC CCT GGG GTA AAA GCA GTG AAA GTG GCA GAT Arg Cys Leu Cys Ile Gly Pro Gly Val Lys Ala Val Lys Val Ala Asp 30 35 40 | 206 |
| ATT GAG AAA GCC TCC ATA ATG TAC CCA AGT AAC AAC TGT GAC AAA ATA Ile Glu Lys Ala Ser Ile Met Tyr Pro Ser Asn Asn Cys Asp Lys Ile 45 50 55 60 | 254 |
| GAA GTG ATT ATT ACC CTG AAA GAA AAT AAA GGA CAA CGA TGC CTA AAT Glu Val Ile Ile Thr Leu Lys Glu Asn Lys Gly Gln Arg Cys Leu Asn 65 70 75 | 302 |
| CCC AAA TCG AAG CAA GCA AGG CTT ATA ATC AAA AAA GTT GAA AGA AAG Pro Lys Ser Lys Gln Ala Arg Leu Ile Ile Lys Lys Val Glu Arg Lys 80 85 90 | 350 |
| AAT TTT TAAAAATATC AAAACATATG AAGTCCTGGA AAAGGGCATC TGAAAAACCT Asn Phe | 406 |
| AGAACAAAGTT TAACTGTGAC TACTGAAATG ACAAGAATTC TACAGTAGGA AACTGAGACT TTTCTATGGT TTTGTGACTT TCAACTTTG TACAGTTATG TGAAGGATGA AAGGTGGGTG AAAGGACCAA AAACAGAAAT ACAGTCTTCC TGAATGAATG ACAATCAGAA TTCCACTGCC CAAAGGAGTC CAACAATTAA ATGGATTCT AGGAAAAGCT ACCTTAAGAA AGGCTGGTTA CCATCGGAGT TTACAAAGTG CTTTCACGTT CTTACTTGTG GTATTATACA TTCATGCATT TCTAGGCTAG AGAACCTTCT AGATTTGATG CTTACAACTA TTCTGTTGTG ACTATGAGAA CATTCTGTC TCTAGAAGTT ATCTGTCTGT ATTGATCTTT ATGCTATATT ACTATCTGTG GTTACAGTGG AGACATTGAC ATTATTACTG GAGTCAGGCC CTTATAAGTC AAAAGCACCT ATGTGTCGTA AAGCATTCC CAAACATTAA TTCATGCAAA TACACACTTC TTTCCCCAAA TATCATGTAG CACATCAATA TGTAGGGAAA CATTCTTATG CATCATTGTTG TTTGTTTTAT AACCAATTCA TTAAATGTAA TTCATAAAAT GTACTATGAA AAAAATTATA CGCTATGGGA TACTGGCAAC AGTGCACATA TTTCATAACC AAATTAGCAG CACCGGTCTT AATTTGATGT TTTTCAACTT TTATTCAATTG AGATGTTTG AAGCAATTAG GATATGTGTG TTTACTGTAC TTTTGTTTT GATCCGTTTG TATAAATGAT AGCAATATCT TGGACACATT TGAAATACAA AATGTTTTG TCTACCAAAG AAAAATGTTG AAAAATAAGC AAATGTATAC CTAGCAATCA CTTTTACTTT TTGTAATTCT GTCTCTTAGA AAAATACATA ATCTAATT | 466 526 586 646 706 766 826 886 946 1006 1066 1126 1186 1246 1306 1354 |

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 94 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Met Ser Val Lys Gly Met Ala Ile Ala Leu Ala Val Ile Leu Cys Ala
 1 5 10 15

Thr Val Val Gln Gly Phe Pro Met Phe Lys Arg Gly Arg Cys Leu Cys
 20 25 30

Ile Gly Pro Gly Val Lys Ala Val Lys Val Ala Asp Ile Glu Lys Ala
 35 40 45

Ser Ile Met Tyr Pro Ser Asn Asn Cys Asp Lys Ile Glu Val Ile Ile
 50 55 60

Thr Leu Lys Glu Asn Lys Gly Gln Arg Cys Leu Asn Pro Lys Ser Lys
 65 70 75 80

Gln Ala Arg Leu Ile Ile Lys Lys Val Glu Arg Lys Asn Phe
 85 90

(2) INFORMATION FOR SEQ ID NO:11:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 813 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 86..544

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

| | |
|--|-----|
| GGGAAGATAAC ATTACACAGAA AGAGCTTCCT GCACAAAGTA AGCCACCAGC GCAACATGAC | 60 |
| AGTGAAGACC CTGCATGGCC CAGCC ATG GTC AAG TAC TTG CTG CTG TCG ATA Met Val Lys Tyr Leu Leu Leu Ser Ile | 112 |
| 1 5 | |
| TTG GGG CTT GCC TTT CTG AGT GAG GCG GCA GCT CGG AAA ATC CCC AAA Leu Gly Leu Ala Phe Leu Ser Glu Ala Ala Arg Lys Ile Pro Lys | 160 |
| 10 15 20 25 | |
| GTA GGA CAT ACT TTT TTC CAA AAG CCT GAG AGT TGC CCG CCT GTG CCA Val Gly His Thr Phe Phe Gln Lys Pro Glu Ser Cys Pro Pro Val Pro | 208 |
| 30 35 40 | |
| GGA GGT AGT ATG AAG CTT GAC ATT GGC ATC ATC AAT GAA AAC CAG CGC Gly Gly Ser Met Lys Leu Asp Ile Gly Ile Ile Asn Glu Asn Gln Arg | 256 |
| 45 50 55 | |
| GTT TCC ATG TCA CGT AAC ATC GAG AGC CGC TCC ACC TCC CCC TGG AAT Val Ser Met Ser Arg Asn Ile Glu Ser Arg Ser Thr Ser Pro Trp Asn | 304 |
| 60 65 70 | |
| TAC ACT GTC ACT TGG GAC CCC AAC CGG TAC CCC TCG GAA GTT GTA CAG Tyr Thr Val Thr Trp Asp Pro Asn Arg Tyr Pro Ser Glu Val Val Gln | 352 |
| 75 80 85 | |

| | |
|--|---------------------|
| GCC CAG TGT AGG AAC TTG GGC TGC ATC AAT GCT CAA GGA AAG GAA GAC Ala Gln Cys Arg Asn Leu Gly Cys Ile Asn Ala Gln Gly Lys Glu Asp 90 95 100 105 | 400 |
| ATC TCC ATG AAT TCC GTT CCC ATC CAG CAA GAG ACC CTG GTC GTC CGG Ile Ser Met Asn Ser Val Pro Ile Gln Gln Glu Thr Leu Val Val Arg 110 115 120 | 448 |
| AGG AAG CAC CAA GGC TGC TCT GTT TCT TTC CAG TTG GAG AAG GTG CTG Arg Lys His Gln Gly Cys Ser Val Ser Phe Gln Leu Glu Lys Val Leu 125 130 135 | 496 |
| GTG ACT GTT GGC TGC ACC TGC GTC ACC CCT GTC ATC CAC CAT GTG CAG Val Thr Val Gly Cys Thr Cys Val Thr Pro Val Ile His His Val Gln 140 145 150 | 544 |
| TAAGAGGTGC ATATCCACTC AGCTGAAGAA GCTGTAGAAA TGCCACTCCT TACCCAGTGC TCTGCAACAA GTCCTGTCTG ACCCCCCATT CCCTCCACTT CACAGGACTC TTAATAAGAC CTGCACGGAT GGAAACAGAA AATATTCACA ATGTATGTGT GTATGTACTA CACTTTATAT TTGATATCTA AAATGTTAGG AGAAAAATTA ATATATTCAAG TGCTAATATA ATAAAGTATT AATAATTAA AAATAAAAAA AAAAAAAA | 604 664 724 784 813 |

(2) INFORMATION FOR SEQ ID NO:12:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 153 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

| |
|--|
| Met Val Lys Tyr Leu Leu Leu Ser Ile Leu Gly Leu Ala Phe Leu Ser .1 5 10 15 |
| Glu Ala Ala Ala Arg Lys Ile Pro Lys Val Gly His Thr Phe Phe Gln 20 25 30 |
| Lys Pro Glu Ser Cys Pro Pro Val Pro Gly Gly Ser Met Lys Leu Asp 35 40 45 |
| Ile Gly Ile Ile Asn Glu Asn Gln Arg Val Ser Met Ser Arg Asn Ile 50 55 60 |
| Glu Ser Arg Ser Thr Ser Pro Trp Asn Tyr Thr Val Thr Trp Asp Pro 65 70 75 80 |
| Asn Arg Tyr Pro Ser Glu Val Val Gln Ala Gln Cys Arg Asn Leu Gly 85 90 95 |
| Cys Ile Asn Ala Gln Gly Lys Glu Asp Ile Ser Met Asn Ser Val Pro 100 105 110 |
| Ile Gln Gln Glu Thr Leu Val Val Arg Arg Lys His Gln Gly Cys Ser 115 120 125 |
| Val Ser Phe Gln Leu Glu Lys Val Leu Val Thr Val Gly Cys Thr Cys |

130

135

140

Val Thr Pro Val Ile His His Val Gln
145 150

What is claimed is:

1. A composition comprising an isolated polynucleotide selected from the group consisting of:
 - (a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:1 from nucleotide 38 to nucleotide 1447;
 - (b) a polynucleotide comprising a fragment of the nucleotide sequence of SEQ ID NO:1 encoding a protein having biological activity;
 - (c) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:2;
 - (d) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:2 having biological activity;
 - (e) a polynucleotide which is an allelic variant of SEQ ID NO:1; and
 - (f) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(e).
2. A composition of claim 1 wherein said polynucleotide is operably linked to an expression control sequence.
3. A host cell transformed with a composition of claim 2.
4. The host cell of claim 3, wherein said cell is a mammalian cell.
5. A process for producing a protein, which comprises:
 - (a) growing a culture of the host cell of claim 3 in a suitable culture medium; and
 - (b) purifying the protein from the culture
6. A protein produced according to the process of claim 5.
7. A composition comprising a protein, wherein said protein comprises an amino acid sequence selected from the group consisting of:

- (a) the amino acid sequence of SEQ ID NO:2; and
- (b) fragments of the amino acid sequence of SEQ ID NO:2;

the protein being substantially free from other mammalian proteins.

8. The composition of claim 7, further comprising a pharmaceutically acceptable carrier.

9. A composition comprising an antibody which specifically reacts with the protein of claim 7.

10. A method for preventing, treating or ameliorating a medical condition which comprises administering to a mammalian subject a therapeutically effective amount of a composition of claim 8.

11. A composition comprising an isolated polynucleotide selected from the group consisting of:

- (a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:3 from nucleotide 52 to nucleotide 2034;
- (b) a polynucleotide comprising a fragment of the nucleotide sequence of SEQ ID NO:3 encoding a protein having biological activity;
- (c) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:4;
- (d) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:4 having biological activity;
- (e) a polynucleotide which is an allelic variant of SEQ ID NO:4; and
- (f) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(e).

12. A composition of claim 11 wherein said polynucleotide is operably linked to an expression control sequence.

13. A host cell transformed with a composition of claim 12.
14. The host cell of claim 13, wherein said cell is a mammalian cell.
15. A process for producing a protein, which comprises:
 - (a) growing a culture of the host cell of claim 13 in a suitable culture medium; and
 - (b) purifying the protein from the culture
16. A protein produced according to the process of claim 15.
17. A composition comprising a protein, wherein said protein comprises an amino acid sequence selected from the group consisting of:
 - (a) the amino acid sequence of SEQ ID NO:4; and
 - (b) fragments of the amino acid sequence of SEQ ID NO:4;the protein being substantially free from other mammalian proteins.
18. The composition of claim 17, further comprising a pharmaceutically acceptable carrier.
19. A composition comprising an antibody which specifically reacts with the protein of claim 17.
20. A method for preventing, treating or ameliorating a medical condition which comprises administering to a mammalian subject a therapeutically effective amount of a composition of claim 18.
21. A composition comprising an isolated polynucleotide selected from the group consisting of:
 - (a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:5 from nucleotide 76 to nucleotide 474;

- (b) a polynucleotide comprising a fragment of the nucleotide sequence of SEQ ID NO:5 encoding a protein having biological activity;
- (c) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:6;
- (d) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:6 having biological activity;
- (e) a polynucleotide which is an allelic variant of SEQ ID NO:5; and
- (f) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(e).

22. A composition of claim 21 wherein said polynucleotide is operably linked to an expression control sequence.

23. A host cell transformed with a composition of claim 22.

24. The host cell of claim 23, wherein said cell is a mammalian cell.

25. A process for producing a protein, which comprises:

- (a) growing a culture of the host cell of claim 23 in a suitable culture medium; and
- (b) purifying the protein from the culture

26. A protein produced according to the process of claim 25.

27. A composition comprising a protein, wherein said protein comprises an amino acid sequence selected from the group consisting of:

- (a) the amino acid sequence of SEQ ID NO:6; and
- (b) fragments of the amino acid sequence of SEQ ID NO:6;

the protein being substantially free from other mammalian proteins.

28. The composition of claim 27, further comprising a pharmaceutically acceptable carrier.

29. A composition comprising an antibody which specifically reacts with the protein of claim 27.

30. A method for preventing, treating or ameliorating a medical condition which comprises administering to a mammalian subject a therapeutically effective amount of a composition of claim 28.

31. A composition comprising an isolated polynucleotide selected from the group consisting of:

- (a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:7 from nucleotide 67 to nucleotide 348;
- (b) a polynucleotide comprising a fragment of the nucleotide sequence of SEQ ID NO:7 encoding a protein having biological activity;
- (c) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:8;
- (d) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:8 having biological activity;
- (e) a polynucleotide which is an allelic variant of SEQ ID NO:7; and
- (f) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(e).

32. A composition of claim 31 wherein said polynucleotide is operably linked to an expression control sequence.

33. A host cell transformed with a composition of claim 32.

34. The host cell of claim 33, wherein said cell is a mammalian cell.

35. A process for producing a protein, which comprises:
 - (a) growing a culture of the host cell of claim 33 in a suitable culture medium; and
 - (b) purifying the protein from the culture

36. A protein produced according to the process of claim 35.

37. A composition comprising a protein, wherein said protein comprises an amino acid sequence selected from the group consisting of:

- (a) the amino acid sequence of SEQ ID NO:8; and
 - (b) fragments of the amino acid sequence of SEQ ID NO:8;
- the protein being substantially free from other mammalian proteins.

38. The composition of claim 37, further comprising a pharmaceutically acceptable carrier.

39. A composition comprising an antibody which specifically reacts with the protein of claim 37.

40. A method for preventing, treating or ameliorating a medical condition which comprises administering to a mammalian subject a therapeutically effective amount of a composition of claim 38.

41. A composition comprising an isolated polynucleotide selected from the group consisting of:

- (a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:9 from nucleotide 75 to nucleotide 356;
- (b) a polynucleotide comprising a fragment of the nucleotide sequence of SEQ ID NO:9 encoding a protein having biological activity;
- (c) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:10;

- (d) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:10 having biological activity;
- (e) a polynucleotide which is an allelic variant of SEQ ID NO:9; and
- (f) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(e).

42. A composition of claim 41 wherein said polynucleotide is operably linked to an expression control sequence.

43. A host cell transformed with a composition of claim 42.

44. The host cell of claim 43, wherein said cell is a mammalian cell.

45. A process for producing a protein, which comprises:

- (a) growing a culture of the host cell of claim 43 in a suitable culture medium; and
- (b) purifying the protein from the culture

46. A protein produced according to the process of claim 45.

47. A composition comprising a protein, wherein said protein comprises an amino acid sequence selected from the group consisting of:

- (a) the amino acid sequence of SEQ ID NO:10; and
- (b) fragments of the amino acid sequence of SEQ ID NO:10;

the protein being substantially free from other mammalian proteins.

48. The composition of claim 47, further comprising a pharmaceutically acceptable carrier.

49. A composition comprising an antibody which specifically reacts with the protein of claim 47.

50. A method for preventing, treating or ameliorating a medical condition which comprises administering to a mammalian subject a therapeutically effective amount of a composition of claim 48.

51. A composition comprising an isolated polynucleotide selected from the group consisting of:

- (a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:11 from nucleotide 86 to nucleotide 544;
- (b) a polynucleotide comprising a fragment of the nucleotide sequence of SEQ ID NO:11 encoding a protein having biological activity;
- (c) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:12;
- (d) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:12 having biological activity;
- (e) a polynucleotide which is an allelic variant of SEQ ID NO:11; and
- (f) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(e).

52. A composition of claim 51 wherein said polynucleotide is operably linked to an expression control sequence.

53. A host cell transformed with a composition of claim 52.

54. The host cell of claim 53, wherein said cell is a mammalian cell.

55. A process for producing a protein, which comprises:

- (a) growing a culture of the host cell of claim 53 in a suitable culture medium; and
- (b) purifying the protein from the culture

56. A protein produced according to the process of claim 55.

57. A composition comprising a protein, wherein said protein comprises an amino acid sequence selected from the group consisting of:

- (a) the amino acid sequence of SEQ ID NO:12; and
- (b) fragments of the amino acid sequence of SEQ ID NO:12;

the protein being substantially free from other mammalian proteins.

58. The composition of claim 57, further comprising a pharmaceutically acceptable carrier.

59. A composition comprising an antibody which specifically reacts with the protein of claim 57.

60. A method for preventing, treating or ameliorating a medical condition which comprises administering to a mammalian subject a therapeutically effective amount of a composition of claim 58.

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

| | | | |
|---|--|--|--|
| (51) International Patent Classification 6 : C12N 15/12, 15/19, C07K 14/47, 14/52 C12N 5/10, A61K 38/17, C07K 16/18 | | A3 | (11) International Publication Number: WO 97/07198 (43) International Publication Date: 27 February 1997 (27.02.97) |
| (21) International Application Number: PCT/US96/12897 (22) International Filing Date: 8 August 1996 (08.08.96) | | (81) Designated States: AU, CA, JP, MX, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). | |
| (30) Priority Data: Not furnished 11 August 1995 (11.08.95) US | | Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i> | |
| (71) Applicant: GENETICS INSTITUTE, INC. [US/US]; 87 CambridgePark Drive, Cambridge, MA 02140 (US). | | (88) Date of publication of the international search report: 24 July 1997 (24.07.97) | |
| (72) Inventors: JACOBS, Kenneth; 151 Beaumont Avenue, Newton, MA 02160 (US). MCCOY, John, M.; 63 Pine Ridge Road, Reading, MA 01867 (US). KELLEHER, Kerry; 50 Hurley Circle, Marlborough, MA 01752 (US). CARLIN, McKeough; 16 Chauncy Street #22, Cambridge, MA 02138 (US). | | | |
| (74) Agent: BROWN, Scott, A.; Genetics Institute, Inc., Legal Affairs, 87 CambridgePark Drive, Cambridge, MA 02140 (US). | | | |

(54) Title: DNA SEQUENCES AND SECRETED PROTEINS ENCODED THEREBY

(57) Abstract

Novel polynucleotides and the proteins encoded thereby are disclosed.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

| | | | | | |
|----|--------------------------|----|--|----|--------------------------|
| AM | Armenia | GB | United Kingdom | MW | Malawi |
| AT | Austria | GE | Georgia | MX | Mexico |
| AU | Australia | GN | Guinea | NE | Niger |
| BB | Barbados | GR | Greece | NL | Netherlands |
| BE | Belgium | HU | Hungary | NO | Norway |
| BF | Burkina Faso | IE | Ireland | NZ | New Zealand |
| BG | Bulgaria | IT | Italy | PL | Poland |
| BJ | Benin | JP | Japan | PT | Portugal |
| BR | Brazil | KE | Kenya | RO | Romania |
| BY | Belarus | KG | Kyrgyzstan | RU | Russian Federation |
| CA | Canada | KP | Democratic People's Republic of Korea | SD | Sudan |
| CF | Central African Republic | KR | Republic of Korea | SE | Sweden |
| CG | Congo | KZ | Kazakhstan | SG | Singapore |
| CH | Switzerland | LJ | Liechtenstein | SI | Slovenia |
| CI | Côte d'Ivoire | LK | Sri Lanka | SK | Slovakia |
| CM | Cameroon | LR | Liberia | SN | Senegal |
| CN | China | LT | Lithuania | SZ | Swaziland |
| CS | Czechoslovakia | LU | Luxembourg | TD | Chad |
| CZ | Czech Republic | LV | Latvia | TG | Togo |
| DE | Germany | MC | Monaco | TJ | Tajikistan |
| DK | Denmark | MD | Republic of Moldova | TT | Trinidad and Tobago |
| EE | Estonia | MG | Madagascar | UA | Ukraine |
| ES | Spain | ML | Mali | UG | Uganda |
| FI | Finland | MN | Mongolia | US | United States of America |
| FR | France | MR | Mauritania | UZ | Uzbekistan |
| GA | Gabon | | | VN | Viet Nam |

INTERNATIONAL SEARCH REPORT

Int'l Application No
PCT/US 96/12897

A. CLASSIFICATION OF SUBJECT MATTER
 IPC 6 C12N15/12 C12N15/19 C07K14/47 C07K14/52 C12N5/10
 A61K38/17 C07K16/18

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
 IPC 6 C12N C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|------------|---|-----------------------|
| X | <p>THE JOURNAL OF IMMUNOLOGY, vol. 154, no. 7, 1 April 1995, pages 3333-3340, XP002032346 MIYAKE ET AL.: "RP105, a novel B cell surface molecule implicated in B cell activation, is a member of the Leucine-rich repeat protein family" see abstract see page 3336; figure 2 see page 3337, left-hand column, paragraph 2 see page 3338; figure 4 --- -/-</p> | 11-19 |

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

* Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *&* document member of the same patent family

2

Date of the actual completion of the international search

5 June 1997

Date of mailing of the international search report

13.06.97

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
 NL - 2280 HV Rijswijk
 Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl,
 Fax (+ 31-70) 340-3016

Authorized officer

Macchia, G

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 96/12897

| C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT | | |
|--|---|-----------------------|
| Category | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
| P,X | DATABASE EMBL ENTRY MM04637 Accession Number W67046, 15 June 1996 MARRA ET AL.: "The WashU-HHMI mouse EST project" XP002032347 see abstract & Unpublished | 21-29 |
| P,X | --- DATABASE EMBL ENTRY MM3831 Accession Number W08383, 27 April 1996 MARRA ET AL.: "The WashU-HHMI mouse EST project" XP002032348 see abstract & Unpublished | 21-29 |
| P,X | --- DATABASE EMBL ENTRY MM93010 Accession Number W17930, 4 May 1996 MARRA ET AL.: "The WashU-HHMI mouse EST project" XP002032349 see abstract & Unpublished | 21-29 |
| P,X | --- DATABASE EMBL ENTRY MM1705 Accession number W11170, 29 April 1996 MARRA ET AL.: "The WashU-HHMI mouse EST project" XP002032350 see abstract & Unpublished | 21-24 |
| P,X | --- DATABASE EMBL ENTRY HS309B01B Accession number D62634, 29 August 1995 FUJIWARA ET AL.: XP002032351 see abstract & Unpublished | 41-44 |
| E | --- WO 97 04097 A (GENETICS INST) 6 February 1997 Seq.ID:1/2 see page 33 - page 34 see page 39 - page 42; claims | 51-60 |
| 2 A | --- WO 95 18826 A (SCHERING CORP ;INST NAT SANTE RECH MED (FR)) 13 July 1995 see page 50 - page 63 --- | 51-60 |
| -/-- | | |

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 96/12897

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|----------|--|-----------------------|
| A | US 4 914 025 A (MANOIL COLIN ET AL) 3 April 1990 see the whole document --- | 1-10 |
| A | JOURNAL OF BACTERIOLOGY, vol. 177, no. 1, 1 January 1995, pages 59-65, XP000560419 ENG MONG LIM ET AL: "IDENTIFICATION OF MYCOBACTERIUM TUBERCULOSIS DNA SEQUENCES ENCODING EXPORTED PROTEINS BY USING PHOA GENE FUSIONS" --- | |
| A | JOURNAL OF CELLULAR BIOCHEMISTRY, vol. 21A, 10 March 1995 - 4 April 1995, page 19 XP002027246 JACOBS ET AL.: "A novel method for isolating eukaryotic cDNA clones encoding secreted proteins" --- | |
| T | US 5 536 637 A (JACOBS KENNETH) 16 July 1996 ----- | |

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 96/ 12897

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.: 10, 20, 30, 40, 50, 60
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although these claims are directed to a method of treatment of (diagnostic method practised on) the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

5 inventions * see continuation-sheet PCT/ISA/210 *

1. As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- The additional search fees were accompanied by the applicant's protest.
- No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/210

1) claims 1-10 totally.

Nucleotide sequence as in Seq.ID:1 encoding polypeptide as in Seq.ID:2, fragments, compositions and potential therapeutical uses.

2) claims 11-20 totally.

Nucleotide sequence as in Seq.ID:3 encoding polypeptide as in Seq.ID:4, fragments, compositions and potential therapeutical uses.

3) claims 21-30 totally.

Nucleotide sequence as in Seq.ID:5 encoding polypeptide as in Seq.ID:6, fragments, compositions and potential therapeutical uses.

4) claims 31-50 totally.

Nucleotide sequence as in Seq.ID:7/9 encoding polypeptide as in Seq.ID:8/10, fragments, compositions and potential therapeutical uses.

5) claims 51-60 totally.

Nucleotide sequence as in Seq.ID:11 encoding polypeptide as in Seq.ID:12, fragments, compositions and potential therapeutical uses.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 96/12897

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|--|------------------|--|----------------------------------|
| WO 9704097 A | 06-02-97 | AU 6712396 A AU 6768596 A WO 9707198 A | 18-02-97 12-03-97 27-02-97 |
| WO 9518826 A | 13-07-95 | AU 1520895 A EP 0733069 A JP 9501572 T | 01-08-95 25-09-96 18-02-97 |
| US 4914025 A | 03-04-90 | NONE | |
| US 5536637 A | 16-07-96 | NONE | |